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Currently the Department of Defense (DoD) does not use exposure biomarkers to measure environmental exposures to chemicals. Blood and urine exposure biomarkers for volatile organic compounds (VOC), selected heavy metals, depleted uranium (DU), and chemical warfare agents are currently available but have not been field tested or validated in military deployments as a tool to document exposures by the DoD. The Military Deployment Human Exposure Assessment Study, a prospective cohort of 46 soldiers deployed to Bosnia, was designed to validate blood and urine exposure biomarkers as a mechanism to document exposures to these chemicals during military deployments. Blood and urine were collected pre-, during, and post deployment. Standard questionnaire was administered, and environmental and occupational monitoring methods were conducted for comparison to the exposure biomarker results. The urine depleted uranium, blood VOC, urine heavy metals, and blood heavy metals results are compared pre-, during, and post deployment and against standard US reference ranges for the same compounds. The results of the study indicate that natural uranium and styrene environmental exposures increased during deployment. Therefore, exposure biomarkers may be a valuable tool in assessing exposures and risk from environmental and occupational chemicals and hence imperative to include in a comprehensive DoD preventive medicine program.

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ABSTRACT

Currently the Department of Defense (DoD) does not use exposure biomarkers to measure environmental exposures to chemicals. Blood and urine exposure biomarkers for volatile organic compounds (VOC), selected heavy metals, depleted uranium (DU), and chemical warfare agents are currently available but have not been field tested or validated in military deployments as a tool to document exposures by the DoD. The Military Deployment Human Exposure Assessment Study, a prospective cohort of 46 soldiers deployed to Bosnia, was designed to validate blood and urine exposure biomarkers as a mechanism to document exposures to these chemicals during military deployments. Blood and urine were collected pre-, during, and post deployment. Standard questionnaire was administered, and environmental and occupational monitoring methods were conducted for comparison to the exposure biomarker results. The urine depleted uranium, blood VOC, urine heavy metals, and blood heavy metals results are compared pre-, during, and post deployment and against standard US reference ranges for the same compounds. The results of the study indicate that natural uranium and styrene environmental exposures increased during deployment. Therefore, exposure biomarkers may be a valuable tool in assessing exposures and risk from environmental and occupational chemicals and hence imperative to include in a comprehensive DoD preventive medicine program.

RESEARCH THESIS for:

The Military Deployment Human Exposure Assessment Study (MDHEXAS): Blood and urine exposure biomarkers as environmental surveillance tools for assessing military personnel exposure to chemicals during deployment to Camp McGovern, Bosnia

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Dissertation submitted to the Faculty of the Department of Preventive Medicine and Biometrics Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of

Doctor of Public Health, 2003

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PREFACE

The Military Deployment Human Exposure Assessment Study: Blood and urine exposure biomarkers as environmental surveillance tools for assessing military personnel exposure to contaminants during deployment to Camp McGovern, Bosnia

Lisa M. May, Dr. P.H., 2003

Dissertation directed by David Cruess, PhD, Uniformed Services University of the Health Sciences; LTC Arthur Lee, USA (Retired), PhD, PE, Uniformed Services University of the Health Sciences; Jack Heller, PhD, USA Center for Health Promotion and Preventive Medicine; LTC Michael Roy, USA, MD, MPH, Uniformed Services University of the Health Sciences; CAPT David Trump, USN, MD, MPH, Uniformed Services University of the Health Sciences; Coleen Weese, MD, MPH, USA Center for Health Promotion and Preventive Medicine.

Statement of the Problem:

A lack of individual exposure information limited the evaluation of exposure-outcome relationships following the Gulf War. Exposure concerns during Operation Enduring Freedom deployments have increased interest in individual environmental and occupational chemical exposure assessment. Currently, deployment assessments are conducted using intermittent ambient air monitoring, occasional focused evaluations based on these results, and post-deployment questionnaire documentation of exposure and/or health concerns. While this strategy is an improvement over prior practice, it has limitations including a reliance on evidence of an acute problem to initiate in depth health evaluations. Exposure biomarkers may have the potential to overcome some of the limitations of current environmental and occupational exposure assessment tools. Exposure biomarkers have not been validated for use in DoD deployments as an exposure assessment tool. Therefore, this research attempts to validate blood and urine exposure biomarkers in these scenarios.

Methods:

The experimental design for this research was a prospective, methodologic cohort. The cohort was identified as an Indiana National Guard unit deploying to Bosnia with greater than 50 persons who ranged in age from 18 to 55, either male or female. The follow up period was the duration of the deployment, approximately 6 months. Biologic samples (blood and urine) were collected and analyzed for chemical exposures in a total of 48 soldiers prior to (February 2002), during (June 2002), and after (August and September 2002) the deployment period. The purpose of this study was to determine if blood and urine exposure biomarkers were capable of determining a difference between the pre-, during, and post-deployment concentrations of toxic chemicals within the blood and urine of deployed DoD personnel, and to field-test exposure biomarkers for chemical agents. To determine over-exposure to toxic chemicals in blood and urine, the US Centers for Disease Control and Prevention (CDC) National Health and Nutrition Examination Survey (NHANES) cohort blood and urine chemical exposure data were used as the external referent comparison group for the study. The NHANES cohort reference range values were used for VOCs and lead in blood, and heavy metals in urine. Pre-, during, and post levels were compared using a paired t-test to determine changes of exposure status in each individual. Volunteers were enrolled in the study if they provided informed consent to participate and were deploying on active duty military status. Questionnaires were completed pre-, during, and post deployment and analyzed for exposure perception and basic demographic data. Additionally, standard air, water, and soil environmental data were gathered, analyzed, and correlated to exposure biomarker results. This research took place in three Phases. Phase I was the site selection,

environmental screening, biomarker selection phase. Phase II was the data collection and sample analysis phase. Phase III was the data analysis and report generation phase.

Results:

Fifty one persons were enrolled in the study pre-deployment. However, only forty six persons completed all three data collection phases of the study (pre-, during, and post deployment) due to the researcher's inability to locate 5 persons throughout all three phases of research. Questionnaire data pre-, during, and post deployment were collected from all 46 persons as well as Informed Consent documentation was obtained from all 51 persons initially enrolled in the study. Cohort demographics include average age, weight, and height. Cohort chemical exposure percentages were presented. The cohort's perception of exposure to passive smoke, fuels, depleted uranium, and chemical warfare agents are reported as percentages and not compared statistically due to the fact that measurement values were adjusted during and post deployment by including an additional response "unknown". The pre-deployment (self-reported questionnaire) data indicate 2.2% of the cohort perceived exposure to depleted uranium. During and post deployment the rates increased to 17.4% and 8.9% respectively. Additionally, 26.1% of the cohort during deployment perceived an unknown exposure to depleted uranium. Translated, approximately one third of the cohort perceived a medium, high, or unknown exposure to depleted uranium when no exposure was verified. In the case of chemical warfare agents, 56.6% of the cohort perceived exposure pre-deployment while during and post deployment respectively 39.1% and 45.6%, with 19.6% of the cohort perceiving an unknown exposure to chemical warfare agents during and post deployment. The pre-deployment questionnaire did not provide the opportunity to give "unknown" as a

response. No known exposure to chemical warfare agents occurred during the deployment.

The MDHEXAS documented VOCs and heavy metals in soil, air, and water but did not specifically sample for uranium due to the fact the UNEP study had been completed. Complete environmental sampling results and personal organic vapor air sampling results are reported.

Minimum, maximum, geometric mean, and confidence intervals of urine uranium, blood VOCs, and blood/urine heavy metals are reported pre-, during, and post deployment to Bosnia. To understand whether the values of environmental chemicals were significantly different during deployment, a paired t-test was performed. The results of the paired t-test for three comparisons are reported: (1) pre-deployment to during deployment, (2) during deployment to post deployment, and (3) pre-deployment to post deployment. Alpha was set at 0.05 and statistical significance was determined with a Bonferroni correction for multiple comparisons. Finally, the statistical difference in pre-deployment to during and post deployment levels were compared to US Standard Reference Ranges of blood and urine environmental chemicals are reported by the CDC in the Second National Report on Human Exposures to Environmental Chemicals (CDC, 2003). The reported reference range geometric mean is reported (CDC, 2003; Ting etal., 1999). The MDHEXAS geometric mean and confidence intervals are also reported. The Task Force 1-151 blood and urine data suggest that exposures were less than the general US population (CDC, 2003).

DEDICATION

This dissertation is dedicated to my husband, Rick. He has been my greatest inspiration. I am deeply grateful to him for his constant support and understanding during this long process and for his enormous confidence in my ability. I also dedicate this work to my son Justin who has understood my frustrations and encouraged me through them, and finally, to my son Noah who gave up his precious time with me but has endured it graciously, forgivingly, and lovingly.

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Personally, I want to express thanks Mrs. Lisa Peacock and her family, Ms. Ninette Sadusky, Mrs. Susan Yialamas, Mrs. Kitty McElyea, Ms. Jean Taylor, CPT Tara Hall, Maj Lisa Webster, Mr. Jaymie Durnan, MAJ Greg Kimm, CDR Gary Hook, LTC Kelly Vest, Dr. David Tribble, LTC Steve Jones, LtCol Art Kaminski, COL Brett Armstrong, and COL David Farrisee for their friendship during this endeavor. Finally, I am grateful to my parents, Mr. Chris Kirk and Mrs. Liz Kirk, my grandmother, Mrs. Helen Kirk, and my brother, Michael Kirk, who are my biggest fans and best friends.

INTRODUCTION

Analyses of health protection efforts during the Gulf War routinely cite the lack of individual exposure information as a limiting factor in clarifying potential etiologies of illnesses that developed post deployment. Concerns raised by military members and Congress during Operation Enduring Freedom deployments to Afghanistan and neighboring nations have identified the need for accurate monitoring, evaluation and documentation of individual environmental and occupational chemical exposures. Valid exposure assessment is crucial to risk assessment, risk management and prevention of illness attributed to such exposures. Many independent groups evaluating Department of Defense (DoD) Force Protection policies and procedures have recommended individual exposure assessment. The DoD has been working diligently to implement a systematic program to evaluate deployment-related individual exposures but has been constrained by the magnitude of the effort. Current efforts have focused on intermittent sampling of ambient air, water and soil. Hazardous levels of chemicals trigger further evaluation and implementation of measures to limit untoward exposures. The purpose of this project was to validate and help define the role of exposure biomarkers (EBs) for deployment exposure surveillance (that will stem from implementing EBs) as part of the comprehensive Environmental and Occupational Health Surveillance program.

ROLE OF THE CANDIDATE

Major Lisa M. May, Doctor of Public Health Candidate, was the Principal Investigator (PI) of this research. As the PI, Major May assumed responsibility to write the research protocol, obtain collaboration and funding, and to gain Institutional Review Board approval for conduct of the study from the Uniformed Services University of the Health Sciences and the US Centers for Disease Control and Prevention. Major May was also responsible for all aspects of data collection, subject treatment and recruitment, as well as records maintenance. This dissertation is submitted to the Faculty of the Department of Preventive Medicine and Biometrics Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Doctor of Public Health, 2003.

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Stand Alone Technical Reports

USACHPPM Deployment Environmental Surveillance Assessment

Battelle Organic Vapor Monitor Report

CHAPTER 1: REVIEW OF THE LITERATURE

1.1 Literature Review Methods

Exposure Biomarkers (EBs) have been recommended for use in DoD by three documents. These are: (1) the 1 February 2002 Joint Chiefs of Staff Memorandum titled, "Updated Procedures for Deployment Health Surveillance and Readiness" (CJCS, 2002); (2) the August 1998 Presidential Review Directive 5 titled "Planning for Health Preparedness for and Readjustment of the Military, Veterans, and Their Families after Future Deployments" (PRD 5, 1998); and (3) the 2000 Institute of Medicine (IOM) Report, "Protecting Those Who Serve" (IOM, 2000). However, exposure biomarkers have not been validated as a human exposure assessment methodology for use in DoD.

In January 2000, the Uniformed Services University of the Health Sciences (USUHS) proposed a collaborative prospective, methodological epidemiologic research study to validate exposure biomarkers for military relevant chemicals during deployments. This study titled, "The Military Deployment Human Exposure Assessment Study (MDHEXAS)" was designed to survey the military population and serve as companion to the Environmental Protection Agency's, "National Human Exposure Assessment Survey (NHEXAS) Study (Robertson, 1999). Additionally, the MDHEXAS was designed with the vision that once completed, the US Centers for Disease Control and Prevention (CDC) would have released their National Report and Second National Report on Human Exposure to Environmental Chemicals in early 2001 and early 2003 (CDC 2001 & 2003). The MDHEXAS was completed in September 2002 and this thesis summarizes the study conception, design, results, and observations. The MDHEXAS

research objectives were to (1) apply and validate the performance of biologically-based exposure biomarker methods in a prospective DoD deployment scenario, (2) select the exposure biomarkers best suited for militarily relevant toxic chemicals, and (3) determine correlations between exposure biomarkers and traditional environmental samples (area and personal). The importance of this research is that it fills the gap for a scientifically validated internal dose measurement method for exposures during DoD deployment activities.

After the need for current research in deployment EBs was supported through current policy documents, a literature review was completed for the most current and relevant deployment-ready exposure biomarker technologies. Additional time was spent researching the literature where EBs were applied in prospective human studies of civilian and military populations. The literature supported the presence of volatile organic compounds in the blood of the general population through the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. This discovery was of extreme importance, because it allowed for a control arm in this prospective deployment cohort for blood volatile organic compound biomarkers.

Some articles were not reviewed based upon relevance to DoD deployment activities and the age of the article. The criteria used to select EBs were:

(1) Sensitive (able to detect chemical when it is present) and specific (able to indicate no chemical when chemical is not present) for common DoD chemicals/exposures,

(2) Simple (non-invasive as possible) collection method,

(3) Validated analysis method,

(4) Availability of environmental and occupational monitoring methods and standards, and

(5) Previously used to statistically determine national reference range levels.

Literature review was split into relevant categories. They were: policy, applied biomarker research, deployment-ready EBs, laboratory procedures for EBs, general EB research needs, health effects and toxicity, and text books. Appendix A lists all acronyms used in this thesis.

Comprehensive literature review continued throughout all phases of this research project. As new literature became available, it was added to the evidence for or against the research methodology outlined in this document. Table 1.1 outlines sentinel publications that directed the design, implementation, and interpretation of the MDHEXAS.

Table 1.1: Sentinel Publications

Category	Publication	Publication
Policy	The Bush Administration's Record of Environmental Progress, October 2002; pg 31; Joint Chiefs of Staff Memorandum, "Updated Procedures for Deployment Health Surveillance and Readiness," February 2003; and Institute of Medicine (IOM), "Protecting Those Who Serve," 2000.	Presidential Review Directive 5, A National Obligation: Planning for Health Preparedness for and Readjustment of the Military, Veterans, and Their Families after Future Deployments, August 1998
Applied Biomarker Research	U.S. Army Environmental Hygiene Agency, Final Report Kuwait Oil Fire Health Risk Assessment, No. 39-26-L192-91, Appendix F, Biological Surveillance Initiative, Aberdeen Proving Ground, MD, 1991	Robertson, GL, et al., The National Human Exposure Assessment Survey (NHEXAS) Study in Arizona – Introduction and Preliminary Results, *Journal of Exposure Analysis and Environmental Epidemiology*, Vol 9, 427-434, 1999
Deployment Ready EBs & Laboratory Procedures	Schramel, P, et al., The Determination of Metals in Urine Samples by Inductively Coupled Plasma-Mass Spectrometry, *Int Arch Occup Environ Health*, Vol. 69, 219-223, 1997. *(Heavy Metals Method)*	Cardinali, FL, et al., The Use of Solid-Phase Microextraction in Conjunction with a Benchtop Quadrupole Mass Spectrometer for the Analysis of Volatile Organic Compounds in Human Blood at the Low Parts-Per-Trillion Level, *Journal of Chromatographic Science*, Vol. 38, 49-54, Feb 2000. *(VOC Method)*
Deployment Ready EBs & Laboratory Procedures	Ejnik, JW, et al., Determination of the Isotopic Composition of Uranium in Urine by Inductively Coupled Plasma Mass Spectrometry, *Health Physics*, Vol. 78, No. 2, 143-146, Feb 2000. *(Uranium Method)* McDiarmid, MA, The Utility of Spot Collection for Urinary Uranium Determinations in Depleted Uranium Exposed Gulf War Veterans, *Health Physics*, Vol. 77, No. 3, 261-264, Sep 1999. *(Uranium Method)*	Barr, DB, Ashley, DL, A Rapid, Sensitive Method for the Quantitation of N-Acetyl-S-(2-Hydroxyethyl)-L-Cysteine in Human Urine Using Isotope-Dilution HPLC-MS-MS, *Journal of Analytical Toxicology*, Vol. 22, 96-103, Mar/Apr 1998. *(Chemical Weapons Method)*
General Research Needs	Bennett, DA, Applying Biomarker Research, *Environmental Health Perspectives*, Vol. 108, No. 9, 907-910, Sep 2000.	WHO Regional Office for Europe, Guiding Principles for the Use of Biological Markers in the Assessment of Human Exposure to Environmental Factors: An Integrative Approach of Epidemiology and Toxicology, *Toxicology*, Vol. 101, 1-10, 1995
Health Effects & Toxicity	McDiarmid, MA., et al., Health Effects of Depleted Uranium on Exposed Gulf War Veterans, Environmental Research Section, Vol. 82, 168-180, 2000	Armed Forces Radiobiology Research Institute, Health Effects of Embedded Depleted Uranium Fragments, 15 Nov 1996
Text Books	Lauwerys, RR., Hoet, P, Industrial Chemical Exposure: Guidelines for Biological Monitoring, Lewis Publishers, Boca Raton, Florida, 1993	Klaassen, Casarett & Doull's Toxicology, 2001

1.2 History of Biomarkers

A fundamental problem in health risk assessment is relating the release of a chemical into the environment with a valid prediction of risk to the human or biological receptor. Adverse health effects to biological receptors begin with the release of a contaminant into the environment; air, water, soil, or food. Subsequent exposure of humans by contact to contaminated environmental media is defined as an external dose, whereas internalization of the contaminated media, via inhalation, ingestion, or dermal absorption, results in an internal dose (Klaassen, 2001). The amount of this internal dose necessary to elicit a response or health effect is referred to as the biologically effective dose (Klaassen, 2001). Traditionally, environmental risk has been assessed by chemical residue determination in samples of environmental media. This traditional method, still in use today, has disadvantages because it is difficult to accomplish and bioavailability is not quantified. Bioavailability is defined as the availability of the chemicals from the environmental matrix to the biological receptor. Depending on the chemical, receptor, and environmental matrix, bioavailability can range from 100 percent to a fraction of a percent (Klaassen, 2001).

Biomonitoring is the use of personal biological samples (biomarkers) to reflect the interaction between a biological system and a potential hazard. There are many types of biological monitoring spanning the continuum from exposure to physiological effect. Biomarkers of exposure are specific chemicals or their metabolites in clinical samples such as blood, urine, saliva, or breath. Biomarkers of effect are metabolites, endogenous substances, or other parameters indicative of a disease process. Biomarkers of

susceptibility measure factors, including genetic, which alter susceptibility to chemical exposure.

Biomarkers of exposure, or exposure biomarkers (EBs), have been used by the DoD in the past. During the Gulf War, the health threat was considered sufficient to initiate biological surveillance due to concern about toxic environmental exposures from the Kuwait Oil Well fires. The volatile organic compound (VOC) blood level ranges for selected exposed personnel in a Kuwait oil well fire study, reflecting the exposures of the average soldier in Kuwait during that time period, were within the reference ranges for the United States established by the Priority Toxicant Reference Range Study (Pirkle et. al., 1995). Careful study design and biological monitoring for VOCs provided important evidence that, during the study period, personnel were not excessively exposed to VOCs as a result of duty in Kuwait. However, these measurements were only captured for a small cohort (28 persons). A CDC companion study of oil firefighters in Kuwait documented elevated (higher than reference range) levels for some VOCs for some firefighters in blood samples obtained on-site while the fires were being fought. Additionally, the 1991 Final Report Kuwait Oil Fire Health Risk Assessment Appendix F Biological Surveillance Initiative (BSI) (USACHPPM, 1991) monitored soldiers biologically. The BSI had two objectives: (1) to quantify exposure to several environmental contaminants by measuring biological markers of exposure and internal dose, and (2) to detect changes in the cohort's well-being through selected objective and subjective measures of health. The BSI measured blood and urine metals, blood VOCs, and sister chromatid exchange frequency. Levels of all contaminants were within national reference ranges (USACHPPM, 1991).

Although biomonitoring was not specifically conducted during the Vietnam War, successful application of EB methods occurred with the Operation Ranch Hand cohort (Michalek et al, 1995). Due to concerns regarding potential health effects associated with dioxin contaminated herbicide Agent Orange, serum dioxin levels were measured and interpreted for dioxin persistence in Operation Ranch Hand veterans 20 years after Vietnam. A limitation of these data is that the participants could have experienced an additional dioxin body burden between their exposure to Agent Orange in the 1960's and the subsequent biomarker study some 20 years later. Due to the persistence of dioxin, this study determined that biological exposure documentation was more conclusive than standard job exposure matrices. The Operation Ranch Hand cohort has been extensively studied for both environmental exposures to dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD), potential exposure-related health effects such as cancer (Ketchum, 1999), diabetes mellitus (Longnecker et al., 2000), and immunologic responses (Michalek et al., 1999). The value of the Operation Ranch Hand cohort to the current research effort is that many of the health effects studies used a biological exposure biomarker to determine exposure. The three studies mentioned above used serum dioxin levels to determine the exposure – outcome relationship.

1.3 Background and Significance

EBs, applied appropriately, provide a mechanism to overcome some of the limits of exposure assessment tools currently employed by DoD. EBs assess combined exposures from inhalation, ingestion, and dermal contact pathways to evaluate the extent of chemical entry into the body, and can provide a mechanism to systematically

document chronic chemical exposures, regardless of whether the environmental exposure levels have exceeded a current reference standard. Therefore, EBs can be used in conjunction with other assessment tools to provide a comprehensive Environmental and Occupational Health Surveillance program that would be able to detect shifts in levels of exposure from garrison to deployed settings.

EBs offer immediate benefits, including: (1) determination of exposure occurrence and uptake of substances into body fluids or organs (2) potential estimate of individual biological dose (3) ability to assess exposures from routes other than inhalation and (4) usefulness in estimating individual health risk when the exposure-effect response relationship is known based on internal dose. Accurate individual exposure measurements allow better epidemiological investigations for acute and chronic exposures to occupational and environmental hazards. Environmental (ambient) monitoring and self-reported questionnaires have limitations in quantifying low level exposures possibly linked to chronic effects and in quantifying individual internal dose by multiple routes. Some of these limitations may be overcome by the appropriate use of EBs.

Current technological advances in EBs allow the measurement of internal dose of certain chemicals at very low levels. In 1999, CDC embarked on applying current EB analytical methods for blood volatile organic compounds (VOCs), urinary pesticides and blood/urine metals in the most recent version of the National Health and Nutrition Examination Survey (NHANES) (CDC, 2003). These measurements reported as national population estimates, are documented in the March 2001 CDC Report, “National Report on Human Exposure to Environmental Chemicals” (CDC, 2001). Currently available

national population estimates provide a comparison levels aiding the interpretation of EB results from samples collected in the field. Therefore, a new paradigm is advancing, one in which exposure is measured as internal dose at the individual or population level.

In policy, EBs are currently recommended or required for use in DoD by the 1 February 2002 Joint Chiefs of Staff Memorandum titled, "Updated Procedures for Deployment Health Surveillance and Readiness" (CJCS, 2002); Presidential Review Directive 5 (PRD-5); the Bush Administration's Record of Environmental Progress (Bush, 2002); and the Institute of Medicine (IOM) Report, "Protecting Those Who Serve" (IOM, 1999). Military operations can be highly mobile and may not afford an opportunity to conduct area sampling. Additionally, the threat of some exposures, such as chemical warfare agents, is increasing. The Institute of Medicine recommended biological samples in addition to environmental samples. In 2002, the Government Accounting Office stated that "for major deployments and deployments in which there is an anticipated threat of chemical exposures, DoD should collect biological samples such as blood and urine from a sample of deployed forces. Samples can be stored until needed to test for validated biomarkers for possible deployment related exposures or analyzed in near real time as needed for high risk groups" (GAO, 2002).

As EBs become more commonplace in occupational settings and public interest in low-level exposures increases, the DoD will need to establish clear criteria for the use of EBs. Criteria have been established for using EBs in evaluating a few, specific environmental exposures to the public. Because CDC laboratories have methods sufficiently sensitive to detect very low levels of some environmental contaminants, the CDC has adopted some general situations warranting EBs. They are:

(1) When a population has identifiable health effects.

(2) When exposure levels (determined by EBs) can distinguish the exposed from the unexposed.

(3) When exposure status using EBs can classify individuals for follow-up in assessment of the relationship between exposure and illness.

(4) Where specific groups may be particularly susceptible.

(5) When effectiveness of an intervention in reducing exposures must be accessed.

The EBs selected (see Table 1.2 and 1.3) for this study were: (1) blood samples for volatile organic compounds analyzed by solid-phase microextraction in conjunction with a benchtop quadrupole mass spectrometer, which measures at the low parts-per-trillion level (Cardinali, 9), (2) blood and/or urine samples for heavy metals (specific to deployment site based upon threat assessment) analyzed by inductively coupled plasma (ICP) mass spectroscopy, (3) urine samples for total and isotopic uranium analyzed by ICP mass spectroscopy, and (4) urine samples for chemical warfare agents analyzed by a new rapid analysis method developed by CDC (CDC, 1999).

Table 1.2: Exposure Biomarkers Selected (Blood and Urine)

Exposure Biomarker	Environmental Monitoring Method/ Exposure Route	Exposure Biomarker Media	Sensitivity/ Specificity Estimates	Persistence in Exposure Biomarker Media	Exposure Biomarker Analysis Technique	NHANES Reference Range	Deployment Risk for Exposure to Agent
Total/Isotopic Uranium	Soil/Ingestion & Inhalation	Urine	94% 97%	12-48 hr Half Life	ICP-Mass Spec	Yes/No	Background/ Armor & Penetrators
Cadmium/ Chromium/Lead[1]	Air & Soil/Ingestion & Inhalation	Blood	94% 97%	Months	ICP-Mass Spec	Yes	Plating, Paint/Bullets
Nerve agent	Air/Inhalation	Urine	94% 97%	Days	Isotope-Dilution GC-MS-MS	No	Weapon
Sulfur Mustard	Air, Soil, Swipe/ Skin Absorption, Inhalation	Urine	94% 97%	Days	Isotope-Dilution GC-MS-MS	No	Weapon
Volatile Organics[2]	Air/Inhalation	Blood	98% 99%	Hours-Days	SPME Mass Spec	Yes	Fuels

Legend: SPME = Solid-Phase Microextraction Technique with Bench top Mass Spectroscopy
Mass Spec = Mass Spectroscopy
ICP-MS = Inductively Coupled Plasma/Mass Spectroscopy
Isotope-Dilution GC-MS-MS = CDC Specific Method using Isotope-Dilution Gas Chromatography-tandem mass spectrometry

[1]Other Heavy Metals EBs meeting Selection Criteria are: Mercury, Cobalt, Antimony, Barium, Beryllium, Cesium, Molybdenum, Platinum, Thallium, and Tungsten

[2]Volatile Organics include the following: Benzene; m, p, and o-xylene; Ethylbenzene; Toluene; 1,1,1-Trichloroethane; 1,4-Dichlorobenzene; 2,5-Dimethylfuran; 2,5-Dimethylfuran; Carbon Tetrachloride; Chloroform; Styrene; t-Butyl Methyl Ether; tert-Butyl Alcohol; Tetrachloroethene; Trichloroethene; Methyl tert butyl ether (MTBE).

1.4 Military Relevant Chemicals, Toxicity, Fate, Transport, and Degradation Products

1.4.1 Toxicity

Toxicity is generally classified into two categories: acute and chronic. The inhalation, skin absorption, and ingestion toxicities listed in the following table indicate primarily acutely toxic effects. The cancer rating denotes the chronic toxic effects. It should be noted that intermediate effects exist and some chronic exposures can produce similar acute effects. Table 1.3 indicates the toxicity of military relevant chemicals and their use in DoD.

The American Conference of Governmental Industrial Hygienists (ACGIH's®) Biological Exposure Indices (BEIs®) are intended as guidelines for the evaluation of

potential chemical exposure health hazards identified in the practice of industrial hygiene. The database for each BEI® recommendation consists of available information on absorption, elimination, and metabolism of chemicals, and on the correlation between exposure intensity and biological effect in workers. BEIs® are available for such chemicals as: acetone, acetyl cholinesterase inhibiting pesticides, benzene, cadmium, chromium, ethyl benzene, lead, styrene (monomer), and xylenes.

Toxicity information is reported from the National Institute of Occupational Safety and Health (NIOSH) Pocket Guide of Hazardous Chemicals, 1996. The cancer rating and BEI® information is reported from the 1996 ACGIH Threshold Limit Values for Chemical Substances and Physical Agents and BEIs®.

Table 1.3: Toxicity of Military Relevant Chemicals

Chemical	DoD Use of Chemical	Inhalation Toxicity Effect	Skin Absorption Toxicity Effect	Ingestion Toxicity Effect	Cancer Rating
Benzene	Fuels	Irrit eyes, skin, nose	Resp sys, gidd, head, nau	Staggered gait, ftg, anor	A1
m-Xylene	Fuels	Irrit eyes, skin, nose	Throat, dizz, excitement	Drow, inco, staggering	
p-Xylene	Fuels	Irrit eyes, skin, nose	Throat, dizz, excitement	Drow, inco, staggering	
o-Xylene	Fuels	Irrit eyes, skin, nose	Throat, dizz, excitement	Drow, inco, staggering	
Ethylbenzene	Fuels	Irrit eyes, skin, muc memb	Head, derm, narco, coma		
Toluene	Fuels	Irrit eyes, nose, ftg, weak	Conf, euph, dizz, head	Dilated pupils, lac, ner	
Methylene Chloride	De-greaser	Irrit eyes, skin, ftg	Weak, Som, li-head, numb	Tingle limbs, nau	A2
1,1,1-Trichloroethane	Solvents	Irrit eyes, nose, CNS	Depres, liver, kidney	Damage, derrm	
1,4-Dichlorobenzene	Solvents	Irrit eyes, nose, liver	Kidney damage, skin	Blisters	
2,5-Dimethylfuran	Combustion				
Carbon Tetrachloride	Solvents	Irrit eyes, skin, CNS	Depres, nau, vomit, liver	Kidney, drow, dizz	A3
Chloroform	Various	Irrit eyes, skin, dizz	Mental dullness, nau, conf	Head, ftg, anes, enlarged	A2
Styrene	Various	Irrit eyes, nose, resp sys	Head, ftg, dizz, conf, mal	Drow, weak, unstead gait	
t-Butyl Methyl Ether	Various	Irrit eyes, skin, nose	Throat, drow, narco		
tert-Butyl Alcohol	Solvents	Irrit eyes, skin, nose	Throat, drow, nacre		
Tetrachloroethene	Solvents	Irrit eyes, nose, throat	Nau, flush face, neck	Verti, dizz, inco, head	A3
Trichloroethene	Solvents	Irrit eyes, skin, head	Vert, vis, dist, ftg, gidd	Tremor, som, nau, vomit	
Nerve Agent	Weapon	Pulm edema	Blister		
Sulfur Mustard	Weapon	Pulm edema	Blister		A1
Total Uranium	Background	Kidney		Cough, chest rales, nau	
Isotopic Uranium (depleted)	Armor, Penetrators	Kidney		Cough, chest rales, nau	
Cadmium*	Plating, Paints	Dust: Pulm edema, dysp Fume: Pulm edema, dysp		Dust: Cough, chest tight	A2
Chromium*	Plating, Paints	Irrit eyes, skin, lung		Fib	A4 CrVI, A1
Lead*	Bullets, Paints	Weak, lass, insom, facial		Pallor, anor	A3

Legend:	anes = anesthesia	anor = anorexia	blisters = blisters
chest rales = chest rales	CNS = Central Nervous System	conf = confusion	cough = coughing
Chest tight = tight chest	depres = depression	Derm = dermatitis	dilated pup = dilated pupils
Dizz = dizziness	dysp = Dyspnea	euph = euphoria	excitement = excited
fib = fibrosis	flush face = flush face	ftg = fatigue	Head = Headache
Gidd = giddiness	inco = incoordination	insom = insomnia	Irrit = irritation
Irrit eyes = irritated eyes	kidney = kidney damage	lac = lacrimation (tears)	lass = Lassitude (weakness)
li-head = light headedness	liver = liver damage	lung = irritated lung	mal = malaise
mental dullness	muc memb = mucous membrane	narco = narcosis	Nau = nausea
Neck = neck ache	nose = irritated nose	Pallor	Pulm edema=pulmonary edema
Resp sys = Respiratory System	skin = irritated skin	som = somnolence (sleepiness)	staggered gait =

			staggered gait
staggering = staggering	throat = irritated throat	tingle limbs = tingling limbs	tremor = tremors
unstead gait = unsteady gait	Vert = vertigo	vis = visual disturbance	vomit = vomitting
A1 = Confirmed Human Carcinogen	A2 = Suspected Human Carcinogen	A3 = Animal Carcinogen	A4 = Not Classifiable as a Human Carcinogen
A5 = Not Suspected as a Human Carcinogen			

These metals are absolutely necessary to analyze due to high military relevance. Other metals may be necessary to analyze for during the deployment. These metals will be chosen based upon the environmental threat assessment and the availability to be detected by the analytical method chosen for heavy metals analysis in this study - Inductively Coupled Plasma-Mass Spectroscopy

Available Biological Exposure Indices (BEIs®) and sampling times are contained below in Table 1.4.

Table 1.4: Biological Exposure Indices (BEIs) of Military Relevant Chemicals

Contaminant	Biological Measure	Time of Sample	BEIs® Value
Benzene	Total phenol in urine	End of shift	50 mg/g creatinine
Benzene	Benzene in exhaled air	Prior to next shift	0.08 to 0.12 ppm
Cadmium*	Urine	Not Critical	5 ug/g creatinine
Cadmium*	Blood	Not Critical	5 ug/L
Chromium*	Urine	End of shift	30 ug/g creatinine
Ethyl Benzene	Urine	End of shift	1.5 g/g creatinine
Lead*	Blood	Not Critical	30 ug/100 ml
Styrene	Blood	End of shift	0.55 mg/L
Toluene	Blood	End of shift	1 mg/L
Trichloroethylene	Blood	End of shift	4 mg/L
Xylene	Urine	End of shift	1.5 g/g creatinine

* These metals are absolutely necessary to analyze due to high military relevance. Other metals may be necessary to analyze for during the deployment. These metals will be chosen based upon the environmental threat assessment and the availability to be detected by the analytical method chosen for heavy metals analysis in this study - Inductively Coupled Plasma-Mass Spectroscopy

The BEIs® and toxicity information give a relatively good picture of the importance and usefulness of studying EBs in DoD deployments for these military relevant, toxic chemicals. BEIs® are based on eight-hour occupational exposures with a 16-hour clearance time between exposure periods. This is unlike the deployment scenario in this study where subjects theoretically could be exposed for 24-hour periods over the deployment period of approximately six months.

1.4.2 Fate and Transport (Lauwerys & Hoet, 1993)

Fate and transport of xenobiotics varies in the environment due to the chemical and physical properties of the contaminant, exposure conditions, heterogeneity of the exposed, and environmental conditions. Fate and transport in the body occurs similarly to fate and transport in the environment. Hydrophilic (water-loving) compounds typically partition into water or compartments in the body containing water (blood). Lipophilic (fat-loving) compounds typically partition into oil or compartments in the

body containing oil (fat). Finally, volatile gases usually partition into the air or compartments in the body containing air (blood and lungs). The following generalize information are provided concerning fate and transport of the named broad categories of xenobiotics.

VOCs: Volatile organic compounds, as organic gases, typically partition into the blood gases and may be degraded or metabolized prior to or as a result of this partitioning. VOCs are thought to be eliminated quickly from the blood because of respiration and cardiovascular system movements. However, VOCs, such as benzene, can cause cell damage through two separate series of events. One is the physiological disposition of benzene, the generation of a series of biologically reactive intermediates and their ability to interact with cells of the bone marrow to initiate toxicity (Klaassen, 2001). The second is the series of events within bone marrow, which results from the interaction of benzene and its metabolites that leads to bone marrow depression and neoplasia (Klaassen, 2001).

Heavy Metals: Heavy metals typically are considered cumulative toxins. Heavy metals are typically excreted by the kidney and can accumulate in the kidney, liver, or bone depending upon the metal. Lead and cadmium will bind to red blood cells and can be detected in the blood, while chromium III will accumulate in the liver, spleen, soft tissues, and bones. Chromium III is recommended for measurement in the urine of humans. The half-lives of various heavy metal compounds vary according to their excretion pathway.

Chemical Agents: Two chemical agents have been selected for study as part of this research effort. They are the nerve agent VX and sulfur mustard. VX, O-ethyl-S-(2-

diisopropylaminoethyl)-methyl phosphonothiolate, is not thought to be as volatile as sulfur mustard, however it is suspected to be more toxic. Nerve agent is a contact hazard to humans. Sulfur mustard, di-2-chloroethyl sulfide, is volatile and a blister agent that is absorbed through the skin.

Total & Isotopic Uranium: Uranium is likely to be oxidized in the body from tetravalent to hexavalent form. Uranium is known to partition into the lungs, kidneys, and bones. Soluble uranium compounds are rapidly eliminated through the kidney with a half-life of between 12-24 hours.

1.4.3 Degradation Products and Excretion Pathways (Lauwerys & Hoet, 1993)

Degradation products of these military relevant chemicals vary in the blood and urine. In general, volatile compounds are excreted through breath in the lungs. Metals are generally compartmentalized in the blood, kidney, and liver. Metals tend to accumulate and remain in the body at their target organ due to their persistent nature (longer half-lives). The following give degradation products suspected for the chemicals of concern in this research study:

VOCs: Ashley found that VOC metabolites are not a good indicator of low-level environmental exposures (Ashley, 1997). This is because low-level VOCs are quickly eliminated from the body into the blood gases or lungs. Higher-level VOC exposures are indicated for analysis of the metabolites of the VOCs because the high-level exposures would be more persistent in the body than the low-level exposures. This persistence could cause some metabolism and in this situation, analysis of metabolites would be

important. Additionally, individual variation has to be considered. Gender, age, body mass index, and the effect of exercise all contribute to individual VOC variation (International Union of Pure and Applied Chemistry, 2000). In studies of individual metabolism of toluene, the literature suggests that concentrations in the blood were higher in females who had exercised within the past eight hours (Baelum, 1990). For this research, it is expected that personnel would be protected against high-level VOC exposures (personal protective equipment or engineering controls). However, during deployments it is possible that personnel are being exposed to low-level VOCs and monitoring and analysis of their blood is warranted. The following lists each metabolic pathways known for the VOCs under study. It should be noted that these metabolic pathways are primarily descriptions of occupational exposures to VOCs. The occupational data are being used to describe degradation because it is the best studied evidence of degradation that exists at this time. VOCs will be sampled and analyzed in the blood because of expected low-level deployment environmental exposures.

Benzene – Phenol is the main urinary metabolite of benzene. In the blood, benzene has been shown empirically to measure at 20 ug/100 ml at the end of a 25 ppm exposure for 2 hours. Additionally, it has been shown to measure 1 ug/100 ml after 15 hours of the same 25 ppm exposure. These studies were part of the evidence to publish a BEI for benzene. (Lauwerys & Hoet, 1993)

Toluene – Cresois, benzylalcohol, benzaldehyde, benzoic acid, and hippuric acid are the main metabolites of toluene. Engstrom, 1976 published a statistically significant correlation between toluene in the blood and urinary hippuric acid at the end of an 8 hour

working day. There are studies supporting correlation between air and blood levels as well. (Lauwerys & Hoet, 1993)

Xylene – The Agency for Toxic Substances and Disease Registry (ATSDR, 1990) has published blood solvent concentrations of m-, p-, and o-xylenes as reaching their maximum at about 150 minutes after onset of exposure and decreased during the latter part of exposure. Xylene can be absorbed through the skin and is metabolized into methylbenzlalcohol, dimethylphenol, methybenzoic acid and methylhippuric acid. The blood concentration of xylenes is thought to be proportional to recent uptake of xylenes. (Lauwerys & Hoet, 1993)

Ethylbenzene- The main metabolites of ethylbenzene are 1-phenylethanol, acetophenone, hydroxyacetophenones, and phenylglyoxylic acid. Blood measurements of ethylbenzene have been studied to show that after exposure to 100 ppm, approximately 0.15 to 0.2 mg/100 ml is detected. In urine, mandelic acid is a better measurement for occupational exposure to ethylbenzene. (Lauwerys & Hoet, 1993)

Methylene chloride – This compound is easily absorbed by the lung where 55-70% is retained and the remaining is partly eliminated in expired air and partly metabolized. The metabolism results in oxidation by P450 cytochrome to carbon monoxide, and in conjunction with glutathione (GSH) to formaldehyde. Carboxyhemoglobin is a good measure for the magnitude of the exposure but is

confounded by exercise, most likely due to increased respiration during exercise. This study will include physical activity information on the questionnaire to capture this confounder data. (Lauwerys & Hoet, 1993)

1,1,1-Trichloroethane – Trichloroethanol and trichloroacetic acid are known metabolites of 1,1,1-trichloroethane. (Lauwerys & Hoet, 1993)

1,4-Dichlorobenzene – This compound is metabolized to dichlorophenol. (Lauwerys & Hoet, 1993)

2,5-Dimethylfuran – There is not much data on 2,5-dimethylfuran metabolites. However, 2,5-dimethylfuran is thought to be detectable in the blood. (Lauwerys & Hoet, 1993)

Carbon Tetrachloride – There is not much data on carbon tetrachloride metabolites. However, carbon tetrachloride is thought to be detectable in the blood. (Lauwerys & Hoet, 1993)

Chloroform – There is not much data on chloroform metabolites. However, chloroform is thought to be detectable in the blood. (Lauwerys & Hoet, 1993)

Styrene – Metabolites of styrene are 4-vinyl phenol, phenylglycol, mandelic acid, benzoic acid, and hippuric acid. In the venous blood, styrene levels are shown to increase with increasing exposures. Exercise seems to confound this relationship similar to methylene chloride. (Lauwerys & Hoet, 1993) The first step in the major metabolic pathway is the formation of styrene 7,8-oxide by the cytochrome P450-medicated

monooxygenase system (IARC, 1994). The major urinary excretion products, mandelic acid, phenylglyoxylic and hippuric acid, are related to styrene glycol, indicating the intermediate formation of styreneoxide to be the major pathway of activation and detoxification of styrene, accounting for more than 85% of the absorbed dose. Saturation of metabolism occurs between 100 and 200 ppm (IARC, 1994). Styrene is thought to accumulate almost exclusively in fat tissue (IARC, 1994).

tert-Butyl Methyl Ether – There is very little data on t-butyl methyl ether metabolites. However, tert-butyl methyl ether is thought to be detectable in the blood. (Lauwerys & Hoet, 1993)

tert-Butyl Alcohol – There is very little data on tert-butyl alcohol metabolites. However, tert-butyl alcohol is thought to be detectable in the blood. (Lauwerys & Hoet, 1993)

Tetrachloroethene – This compound is metabolized to trichloroacetic acid. There are few studies of biomarkers of tetrachloroethene. However, studies indicate that blood is probably the best marker. The concentration in blood is thought to reflect the most recent exposures up to 16 hours post exposure similar to xylene exposure. (Lauwerys & Hoet, 1993)

Trichloroethene – Trichloroethene is metabolized to trichloroethanol, trichloroacetic acid, monochloroacetic acid, and dichlorovinylcysteine. These metabolites can be detected in whole blood and plasma. Concentrations exist in blood

which reflect the most recent exposure when blood is taken during exposure up to 16 hours post exposure. (Lauwerys & Hoet, 1993)

Heavy Metals:

Cadmium - Cadmium is a cumulative toxin with an approximate half-life of greater than 10 years. In the blood, 70% of cadmium is bound to the red blood cells. Cadmium tends to accumulate in the kidney and liver with about 50% of the total body burden found in these organs. It is known that the total body burden of cadmium for smokers is twice that of non-smokers. Therefore, while blood or urine biomarkers of exposure are acceptable, smoking data per participant is extremely important to capture. (Lauwerys & Hoet, 1993)

Chromium - Total chromium speciates to a hexavalent and trivalent form. In the body, the lung, gastrointestinal (GI) tract, and intact skin absorb hexavalent chromium (Chrome VI). The half-life of chrome VI in urine is about 15 to 41 hours. However, Chrome VI is difficult to detect in the urine, indicating that it is rapidly reduced before excretion. Detailed kinetic studies show half-lives of 7 hours, 15 to 30 days, and 3 to 5 years. Trivalent chromium (Chrome III) is typically accumulated in the liver, spleen, soft tissues, and bones. As with Chrome VI, Chrome III partitions into three compartments. These have respective half-lives of 0.5 to 12 hours, 1 to 14 days, and 3 to 12 months. Therefore, urine EBs are recommended to document Chrome III exposures and back calculate Chrome VI suspected exposures (Lauwerys & Hoet, 1993)

Lead – Lead is a cumulative toxin absorbed by the lungs and the GI Tract. In the blood, lead binds to the red blood cells and is not degraded. The first compartment that lead partitions into is the blood, the second is the soft tissues, and the third is the bone. The respective half-lives are 35 days, 40 days, and 20 years. Lead is typically excreted through the kidney, bile, GI, hair, nail, and sweat. On a group sampling basis, there is satisfactory correlation between lead in blood and urine. Therefore, blood or urine biomarkers of exposure are acceptable. (Lauwerys & Hoet, 1993)

Chemical Warfare Agents:

Nerve Agent (VX) - The chemical name for VX is O-ethyl-S-(2-diisopropylaminoethyl)-methyl phosphonothiolate. Specific metabolites of VX in serum are ethyl methylphosphonic acid and 2-(diisopropylamino-ethyl)methyl sulfide (Tsuchihashi, 1998).

Sulfur Mustard (HD) – The chemical name for HD is di-2-chloroethyl sulfide. Sulfur mustard hydrolyze rapidly in the body to form hydrochloric acid (HCl) and thiodiglycol (ATSDR, 2003).

Total & Isotopic Uranium: Uranium is known to oxidize in the body from the tetravalent to the hexavalent form. Soluble uranium compounds are rapidly eliminated through the kidneys. Insoluble uranium can be retained in the lungs. The half-life of uranium in the body is between 12-24 hours. The main excretion occurs through the urine and therefore, urine will be analyzed by ICP-MS for uranium.

1.5 Current Exposure Biomarker Methods

Only current field-tested EBs were considered for this study because of the need to validate these methods in the DoD deployment setting. The other EB media that were available at the time of this study were: hair digested and analyzed by inductively coupled plasma mass spectroscopy for heavy metals; sebaceous tissue; cheek swabs; and serum. All were considered as potential EB media. The EB methods chosen for validation in DoD deployments were:

1. Blood analysis using solid-phase microextraction in conjunction with a bench top quadrupole mass spectrometer for the analysis of volatile organic compounds (VOCs) in human blood at the low parts-per-trillion level (Cardinali, 2000).

2. Urine analysis using ICP-MS for the analysis of total uranium, isotopic uranium, nerve agents, sulfur mustard, cadmium, chromium, and a suite of other heavy metals.

3. Blood analysis using ICP-MS for the analysis of lead (NHANES III, 1998).

In this study, the focus was on these EBs because they have been field tested and are technologically advanced enough to implement in a full-scale DoD project. National reference ranges are available for all chemicals except nerve agent, sulfur mustard, isotopic uranium, and chromium (Schramel et al., 1997).

1.6 US Population-Based Exposure Biomarkers

The Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994 collected blood, urine, serum, and other biological measurements on 39,994 persons greater than two months old; 20,050 (of the 39,994) were adults. Exposure biomarker data contained in the NHANES III data set are VOC measurements in blood, lead measurements in blood, and cadmium measurements in urine. The data set is useful for national reference ranges in the general U.S. population for VOCs, lead, and cadmium. The VOC measurements were analyzed in blood using Purge and Trap Gas Chromotography with Mass Spectroscopy (GC-MS). The detection limits using the purge and trap method are similar enough to allow direct comparison of the results to the new analytical method, the Solid-Phase Microextraction (SPME) Technique with Bench Top Mass Spectroscopy. CDC switched their analytical method to the SPME-MS primarily so that their lab can obtain higher throughputs of analysis. The Purge and Trap method could only analyze four samples per day per GC-MS, and the SPME-MS allows analysis of almost 15 samples per day per MS. NHANES III also maintains a military specific data set for these biomarkers. The military specific data set will be investigated, but will not be used as the general U.S. population reference ranges. Full data sets are available from the Centers for Disease Control and Prevention at their web site. The CDC released these data in the January 2001 National Center for Environmental Health report, “National Report on Human Exposure to Environmental Chemicals.”

In addition to these data sets, NHANES III maintains a sera repository for future testing. The overall goal of the NHANES is to document and monitor the health and

nutrition status of the general U.S. population. Specifically, relationships between EBs and trends in risk behavior and environmental exposures are addressed. NHANES also establishes a national probability sample of genetic material for future genetic testing. NHANES into the 21st Century is another phase of the NHANES project. This follow on NHANES began in April 1999 and is a continuous survey visiting 15 U.S. locations each year. The survey samples approximately 5,000 persons annually. This new and improved NHANES monitors for VOCs in blood using the biomarker method being tested in this research protocol. The data generated from the survey will be linked to related Federal Government surveys of the general U.S. population, such as the National Health Interview Survey (NHIS). In January 2001, the U.S. Department of Agriculture Continuing Survey of Food Intake by Individual (CSFII) released a study merged with the NHANES. The National Food and Nutrition Survey (NFNS) combined with other surveys provide comprehensive information on health and nutrition characteristic of the U.S. general population.

The benefits of the NHANES III and NFNS data are immense to this biomarker study. Previously, researchers needed to use the entire 4-6 year sample to make even the broadest statistical estimates, because the data were only representative of the entire population if one used the entire sample period. The new NFNS design allows increased flexibility in survey content, as well as providing national reference ranges of VOCs in blood, lead in blood, and cadmium in urine. This biomarker study may provide evidence for further national probability sampling of the general U.S. population for other chemicals typical to environmental exposures. Due to the availability of the NHANES

III data set, analytical methods chosen for the deployment biomarkers (VOCs in blood, lead in blood, cadmium in urine) will be those used in the NHANES III study. Other contaminants of concern will be analyzed using the most sensitive analytical method available.

In January 2003, CDC released their second report on US population-based exposure biomonitoring of environmental chemicals in the National Center for Environmental Health report titled, "Second National Report on Human Exposure to Environmental Chemicals." (CDC, 2003) This report details the levels of approximately 35 additional chemicals in the blood and urine of participants in the NHANES. This allows for comparisons of military members in the MDHEXAS to the general public for additional VOCs and metals. Both the First and Second National Report on Human Exposure to Environmental Chemicals can be found on the CDC website: www.cdc.gov/exposurereport/. Finally, the EPA in collaboration with the CDC conducted a study of residents in the Southwest US that tested environmental monitoring with extensive questionnaire data gathering and exposure biomonitoring. Preliminary results were released for this study in 1999 (Robertson, 1999). The title of the study is "The National Human Exposure Assessment Survey (NHEXAS) (Robertson, 1999). These studies influenced the study design of the MDHEXAS and provide important preliminary data for interpretation of study results.

1.7 The Need for DoD Population-Based Exposure Biomonitoring

As stated previously, exposure Biomarkers have been recommended for use in DoD. However, exposure biomarkers have not been validated as a human exposure assessment methodology for use in DoD. President Clinton issued an Executive Order in November, 1993 which established Presidential Review Directive 5, "A National Obligation: Planning for Health Preparedness for and Readjustment of the Military, Veterans, and Their Families after Future Deployments." One of the goals of this document specifies to "strengthen the national strategy to protect and defend military service members from warfare and terrorism with Chemical and Biological Weapons warfare (CBW) agents." Other goals specify that DoD will "implement an effective health risk communication strategy" and requires DoD to "expand research in human biological monitoring to increase the number of chemicals that can be assessed and improve the analysis time and data interpretation." The second policy document reviewed was the Institute of Medicine 1999 report titled, "Strategies to Protect the Health of Deployed U.S. Forces: Medical Surveillance, Record Keeping, and Risk Reduction." This document specifically states that, "improvements in medical surveillance and record keeping after deployments will be needed to note any long-term effects from environmental exposures." Finally the IOM Report requires the use and testing of EBs for DoD force health protection. EBs are one of the approved methods for documenting environmental exposures specific to the individual deployed. However, more research is needed to better classify potential uses of EBs and hence the purpose of this project.

CHAPTER 2: RESEARCH METHODS & STUDY DESIGN

2.1 Research Questions and Technical Objectives

After conducting the literature review, three primary research questions emerged. They were:

(1) Is it possible to use exposure biomarkers (EBs) to measure military relevant chemicals in the blood and/or urine of DoD personnel in the deployment setting?

(2) If it is possible, which EBs perform the best in DoD deployments?

(3) Finally, how do blood volatile organic compound (VOC) levels (EBs) correlate with environmental and/or occupational monitoring levels?

From the above research questions, it was determined that a deployment field test of exposure biomarkers was necessary and the following technical objectives were developed:

TECHNICAL OBJECTIVE #1 -

Demonstrate that blood and urine EBs collected pre-, during, and post deployment for certain toxic environmental chemicals common in DoD deployment environments are sensitive (able to detect chemical when it is present) and specific (able to indicate no chemical when chemical is not present) enough to detect differences in individual internal exposures.

TECHNICAL OBJECTIVE #2 -

Based upon the results of technical objective #1 and the performance of EBs during the field test, select EBs that perform the best to document individual internal exposures during deployments to DoD relevant chemicals.

TECHNICAL OBJECTIVE #3 -

Describe correlations between internal exposure biomarker monitoring results and external environmental monitoring results.

2.2 Specific Aims and Research Hypotheses

From the above research questions and technical objectives, the following specific aims were generated:

Specific Aim 1 - Field test blood and urine biomarkers for use in DoD deployment missions.

Specific Aim 2 - Select the EBs relevant for these missions.

Specific Aim 3 – Define correlations between EBs and environmental samples for application to health risk assessment.

To meet the three research specific aims stated above, three hypotheses were generated.

(1) HYPOTHESIS #1: blood and urine EBs have sufficient accuracy that a difference (null set at zero difference) in environmental exposures can be detected between pre-, during, and post levels of specific chemicals in deployed DoD personnel while considering ranges in the general population (NHANES III national reference ranges of VOCs in blood, metals in blood and/or urine, and uranium in urine). The expected result was that the analytical methods associated with the EBs evaluated by this research were sensitive and specific enough to detect differences between pre-, during, and post levels of volatile organic compounds, total and isotopic uranium, chemical agents, and heavy metals while considering ranges in the general population (NHANES

III national reference ranges of VOCs in blood, metals in blood and/or urine, and uranium in urine) and small sample size.

(2) HYPOTHESIS #2: blood and urine EBs selected for study combined with current environmental sampling add significantly to the quality of exposure data as compared to current questionnaire and environmental sampling levels alone to assess environmental health risk to deployed DoD personnel. To accomplish this goal, standard questionnaire data will be collected at all three phases of data collection. The expected outcome was that blood and urine EBs add significantly to the evidence of exposure used to assess environmental health risks during deployment. This was primarily because EBs measure specific individual internal dose, rather than extrapolating internal dose from a series of environmental measures.

(3) HYPOTHESIS #3: blood VOC measurements (EBs) correlate linearly with occupational breathing zone measurements of VOCs. The expected outcome was that EBs would correlate linearly with the occupational breathing zone measurements collected during the field test. This portion of research was limited to VOCs due to the fact that occupational breathing zone measurements require different collection techniques per grouping of chemicals (metals, radiologic, VOCs). Additionally, individual breathing zone measurements are difficult to collect during a deployment. The individual breathing zone sample collection technique used in the field test was Organic Vapor Monitors (OVMs) which are simply worn on the individual’s lapel. OVMs passively collect the air around the individual therefore do not require an electronic pump to pull air through a filter.

2.3 Research Design and Methods

2.3.1 Overview of Design

The experimental design for this research was a prospective, methodological cohort. The cohort was identified as an Indiana National Guard unit deploying to Bosnia with greater than 50 persons who ranged in age from 18 to 55, either male or female. The follow up period was the duration of the deployment, approximately 6 months. The goal of this study was to determine if blood and urine EBs were capable of determining a difference between the pre-, during, and post-deployment concentrations of toxic chemicals within the blood and urine of deployed DoD personnel, and to field-test EBs for chemical agents.

2.3.2 Questionnaire Design

The questionnaire was designed for two purposes: (1) to collect demographic data, and (2) to document environmental exposure perception. Questionnaire design was conducted using standard survey design techniques and included a set of cognitive interviews to validate the survey instrument (Memon, 1999). The cognitive interview took place in October 2001 at an ordnance brigade on Aberdeen Proving Ground, MD. The cognitive interview required that the volunteer simply answer questions regarding the survey instrument in a truthful and detailed manner. There were no risks to the volunteers and the only benefit received was food (juice and donuts) provided by the Major May during the interviews. The personnel who completed the cognitive interviews were not participating in the field test. Their only role was to critique the questionnaire. The complete cognitive interview explanation is contained in Appendix D. Three

different (pre-, during, and post) questionnaires were developed and tested prior to administration during deployment to Camp McGovern, Bosnia.

Questionnaires were evaluated by six faculty members and the Institutional Review Board (IRB) of the Uniformed Services University of the Health Sciences (USUHS) as well as the IRB at the Centers for Disease Control and Prevention (CDC). The pre-deployment questionnaire was designed to capture work, home, and hobby chemical exposures. During and post deployment questionnaires were designed to capture perceived environmental and occupational exposures related to military deployment. Complete questionnaires are attached in Appendix E. A separate document was developed to address the cognitive interviews and was sent to the USUHS IRB in mid-July, 2001 (Appendix D & E). The questionnaires did not contain any identifying information. The questionnaires were tracked by a numeric specific to the individual. Major May was the sole keeper of the key identifying the number to the individual.

2.3.3 Study Subjects

Cohort entry criteria were: (1) members of a military unit with greater than 50 persons, (2) age ranging from 18 to 55, (3) either male or female, and (4) serving in Active Duty military status. There were no exit criteria. Volunteers were enrolled in the study if they provided informed consent to participate and were deploying on active duty military status. All volunteers were given $30 once blood and urine were collected at all three phases of the deployment process. The DoD definition of deployment is any current or past event or activity that relates to duty in the armed forces that involves an operation, location, command, or duty that is different from the military member's normal duty assignment (DoD, JP 1-02, 1994). Neither location nor cohort individuals

were selected due to expected exposures or over-exposures to environmental chemicals. The cohort was selected based upon availability of members to volunteer for the study. The follow-up period was the duration of the deployment, approximately 6 months. Biologic samples (blood and urine) were collected and analyzed for chemical exposures in a total of 46 soldiers prior to (February 2002), during (June 2002), and after (August and September 2002) the deployment period. To determine the magnitude of exposure to toxic chemicals, the CDC National Health and Nutrition Examination Survey (NHANES) cohort blood and urine chemical exposure data reported in the CDC National Report and Second National Report on Human Exposure to Environmental Chemicals (CDC 2001 & 2003) were used as the external referent comparison group for the study. The NHANES cohort reference range values were used for VOCs and lead in blood, uranium in urine, and heavy metals in urine.

2.3.4 Statistical Measures

To determine a difference between pre-, post, during, and national reference range chemical levels in the blood and urine, sample size was computed using the N-Query software program. The sample size required to determine statistically significant differences in chemical levels was 34 persons. This figure was computed using an effect size of 0.5 as recommended by Cohen (Cohen 1998), alpha of 0.05, beta of 0.05 (power 95%) for a standard two-sided t-test. Fifty persons were required per each set of blood and urine collected to account for an expected 20% loss to follow up at each stage of sampling. Therefore, a total of 150 blood and urine samples (3 collections of 50 samples each) were planned for each set of analyses (urine uranium, blood VOCs, urine heavy metals, and blood heavy metals). The one exception was the urine chemical warfare

agent test which required collection of only 50 urine samples during deployment not pre-, nor post deployment. Samples were collected on the individual level for comparison on the individual level. The method used to calculate sample size was conservative and allowed for multiple comparisons (pre- to during, pre- to post, during to post, and the highest of the pre-, during, post levels to the national reference ranges). It should be noted that Cohen's method does not depend on the units of the observations. Effect size was based on the ability to detect one-fifth of a standard deviation around the mean, which Cohen defines as a strong measure.

Pre-, during, and post levels were compared using a paired t-test to determine changes of exposure status in each individual. Geometric mean was calculated for all biological (EB) measurements. The geometric mean was calculated by taking the log of each concentration, then calculating the mean of those log values, and finally, taking the antilog of that mean (the calculation can be done using log base e or log base 10). Geometric mean provides a better estimate of central tendency for data that are distributed with a long tail at the upper end of the distribution. This type of distribution is common when measuring environmental chemicals in blood or urine. The geometric mean is less influenced by high values than is the arithmetic mean (CDC 2003).

2.3.5 Pretest Activities

The initial step of this research protocol was field test site selection and continued literature review to refine the list of toxic chemicals listed in Table 1.2 and Table 1.3. An additional field test site would strengthen the results of this study and was suggested by the US Army Center for Health Promotion and Preventive Medicine. However, funding

did not permit a second site. The field test site selection was based upon the following criteria:

(1) current deployment area – Iraq, Afghanistan, SW Asia, Kosovo, or Korea,

(2) command approval from CINC Surgeon, Battalion Commander, and Battalion Surgeon,

(3) logistic routes available,

(4) unit exceeding 50 persons,

(5) deployment greater than 30 days,

(6) efficient data available on potential exposures matching with exposure biomarkers,

(7) history of environmental sampling in the area.

The site identified as meeting these criteria was Camp McGovern, Bosnia. Major May worked with the US Army Center for Health Promotion and Preventive Medicine (USACHPPM) to gather the correct clearances and US Army support for this research at Camp McGovern, Bosnia. Continued literature search focused on gathering historical information on DoD relevant toxic chemicals, EBs, and methods to assess the use of biomarkers, current field-deployable biomarkers, and potential comparison groups.

Using the literature review data, laboratory specific sampling criteria, and the information obtained from site-selection, the sampling plan/protocol (See Appendix F) was developed to outline the exact steps required to collect, and analyze EBs and concurrent environmental monitoring.

2.3.6 Materials and Resources

Laboratory analyses were conducted at the CDC National Center for Environmental Health (NCEH), USACHPPM, and the Armed Forces Institute of Pathology (AFIP). CDC maintained the bench top quadrupole mass spectrometer, which measures VOCs in blood at the low parts-per-trillion level and the laboratory equipment to complete the chemical agent screen. AFIP and USACHPPM maintain ICP mass spectrometers, which measures total and isotopic uranium in urine and heavy metals in blood and/or urine. Extractions and creatinine urine adjustments for heavy metals and uranium were conducted at the AFIP laboratory.

This research project was a field-test requiring travel to a foreign land and communication with colleagues at CDC, USACHPPM, USUHS, and AFIP. USACHPPM provided six technical personnel to travel with and assist Major May in data collection activities. Pre-deployment, three environmental scientists supported data collection activities. During deployment, one environmental engineer and three environmental scientists traveled to Bosnia in support of this research. Post deployment, one environmental engineer and one environmental scientist supported data collection at Ft. Dix. Analyses, manpower, and travel costs are outlined in the detailed research budget (Appendix B). Funds were provided by USACHPPM and USUHS.

Clinical supplies were provided by CDC, USACHPPM, AFIP and USUHS. These included vacutainers, urine cups, coolers, ice packs, needles, gauze, band aids, and gloves among other incidentals.

2.3.7 Ethical Considerations

The MDHEXAS is a research study on human subjects and therefore required full IRB approvals by both USUHS and CDC prior to the start of the research. The IRB

approved both the research protocol, sampling plan, and questionnaires. Appendix G outlines the USUHS and CDC IRB approved Informed Consent Form. In addition to meeting the IRB, Major May discussed this research protocol in detail with Dr. Henry Mannix, USUHS General Counsel. From that meeting, it was decided that all biological samples be destroyed after analysis. Also determined at that meeting was that each study participant be informed of the exact use of their blood and urine sample, and be given the opportunity to obtain the results of this research study. Additionally, study participants must be informed that this project is a research effort and only general results can be sent to each participant. The general results will be discussed completely with Dr. Coleen Weese, MD, MPH, USUHS Advisory Committee Member, prior to release. Participants must be informed that specific, individual interpretation of the study results can only be completed by their physician. Prior to data collection, a briefing was provided to the unit commander, Lieutenant Colonel Carr who was required to complete a research ethics training. He also provided a letter of approval to the USUHS IRB. Finally, fact sheets and a briefing were provided to all potential study volunteers. To ensure that no one was pressured into participating in the research, an Ombudsman (Unit Chaplain) was made available to unit members. Participants were given the right to withdraw from the study at any time without recourse.

2.3.7.1 Risks and Benefits Assessment

There were two identified risks to persons enrolling in this study. The first risk to the individual in this research was an adverse reaction to a blood draw. To mitigate this risk, a licensed DoD health care practitioner was available to volunteers during all research-related activities. The second risk was that the participant had the opportunity to

request the general results of the blood and urine samples analyzed for various environmental contaminants. This was risky because at project start, there were few known methods to interpret these results. To mitigate this risk, the individual was directed toward their physician. However, this knowledge could cause undue stress to the participant. The benefits from this study to the research subject were: (1) payment of $30 to each volunteer who donated three blood samples, (2) participation in a scientifically based method of health surveillance during deployments. It should be noted that participants were paid for the donation of blood samples according to DoD regulation. Medical care was guaranteed in the event of an adverse reaction.

The risk to the DoD was that individuals may be concerned over the general test results reported to them from the study. This notification procedure could cause the individual to panic and approach the press. To minimize this risk, this research was briefed in complete to each study participant by Major May. She developed a fact sheet describing the study and the risks/benefits to each participant. Additionally, Major May coordinated with the Battalion Surgeon and Major Command to alleviate any negative feedback from the study. The DoD benefited from this field test through the knowledge of which EBs would be useful during deployments. Additionally, the DoD had an opportunity to complete health risk communication during this study, which will enable future use of this technique during deployments to be better understood.

2.3.7.2 Contingency Planning

If an exposure has been identified that was over a risk level identified in federal law by the Occupational Safety and Health Administration (OSHA) or the Environmental Protection Agency (EPA), persons would be identified and protective measures would be

put in place to ensure that the subject was treated and/or protected from future exposures. It must be noted that EBs are not intended for measuring acute exposures to occupational or environmental chemicals. EBs document chronic exposures to environmental chemicals and therefore it would not be expected that abatement procedures would be implemented during this field test.

2.3.7.3 Results Reporting

The urine/blood heavy metals results were not available at the time of this thesis writing. Therefore, the following explains the plan for complete release and reporting of results to study participants. All general blood and urine results will be reported to those study subjects who requested results on the informed consent form. Personnel will obtain the general sampling results, the average of the study sample (n=50), the NHANES reference ranges where available, and a fact sheet (Agency for Toxic Substances and Disease Registry Approved Fact Sheet for each Chemical) explaining how to interpret the results presented to them. The data summary/fact sheet will explain the results, limitations, and relevance to the participant in a clear, concise, general manner. The data summary/fact sheet will make broad, general interpretations of the data collected in this research effort. Study subjects are to be informed that a more specific, individual interpretation could only be obtained through their personal physician. Results will be mailed to the home of residence collected during the study. A complete briefing of research results is scheduled at a National Guard weekend drill in December of 2003.

2.3.7.4 Modification of the Protocol

If the research protocol was modified in any way from the original having IRB approval, it would have been sent to the USUHS and CDC IRB for a continuing review

and additional approval. The research protocol was not modified from its original version. However, the research protocol required an extended approval due to the time required for analysis.

2.3.7.5 Roles and Responsibilities

The study personnel volunteering for this research were required to provide blood samples at three different times. During each blood draw, three tubes of blood were collected with one needle stick to the volunteer. Therefore, each volunteer provided nine tubes of blood over the entire field test. Additionally, volunteers provided urine at three separate times. One urine sample was provided pre-deployment. Two urine samples were provided during deployment, and one sample post deployment. Questionnaires were completed pre-, during, and post deployment as well. As part of the informed consent process, study subjects were given the opportunity to withdraw from this study at any time and were asked if they wanted to continue in the study prior to any collection of biological specimens.

2.3.7.6 Confidentiality

All data and medical information obtained on volunteers of this research were considered privileged and held in confidence; subjects were not identified in any presentation of the results. Complete confidentiality was not promised because information bearing on health may be required to be reported to appropriate medical or command authorities. Informed consent was obtained from every study participant prior to sampling and questionnaire administration. A coding system for human subjects was developed and maintained by Major May. Subjects were numbered with an identification code, which was not identifiable by anyone but Major May. The questionnaires

contained the code number and Major May maintained a master key listing the volunteers' name, social security number, address, study name, and study dates. All samples donated in this study were used for this study only and were destroyed after analysis was complete for the specific compounds detailed in this protocol.

The results of this research study were given to the USACHPPM and may be asked for by the US Department of Health and Human Services. None of the information given to these people will contain names or other information linking any results to you specifically. Records from this study do not use names or other personal identifiers. Information and other records related to this study were kept private, accessible only to those persons directly involved in conducting this study and members of the USUHS IRB and other Federal agencies who provide oversight for human use protection.

All questionnaires and forms were kept in a restricted access, locked cabinet while not in use. The questionnaires were numbered to maintain anonymity and do not contain any identifying information. Only the project officer in charge has access to the code. However, under federal law, a military member's confidentiality cannot be strictly guaranteed. To enhance privacy, data from questionnaires were entered into a database in which individual responses were not identified. After verification of the database information, the hard copies of the questionnaires containing identifiers were shredded. It is important to reiterate that all biological samples, blood and urine, were destroyed after the analysis was completed. The biological samples were not used for anything other than the determination of volatile organic compounds in blood, metals in blood and urine, uranium in urine, and chemical warfare agents in urine.

2.4 Data Collection

Data collection pre-, during, and post deployment was completed on 13 September 2002. Soldiers deployed to Bosnia from the Indiana National Guard, Task Force 1-151, participated in this study and provided blood and urine samples for analysis according to the exposure biomarker protocol described herein. The test location for pre-deployment sampling was identified as Fort Dix, New Jersey. Fort Dix is a mobilization center which processes soldiers for deployment. The typical type of processing conducted at a mobilization center includes medical evaluation, financial assistance, and training requirements. During protocol design, it was determined that the pre-deployment sampling for the MDHEXAS should occur at a mobilization site where blood and urine sampling was already occurring.

2.4.1 Questionnaire

The questionnaire was given to all 46-study participants pre-, during, and post deployment. The questionnaire was scientifically developed using literature review. The questionnaire was designed for two purposes: (1) to collect demographic data, and (2) to document environmental exposure perception. Questionnaire design was conducted using standard survey design techniques and included a set of cognitive interviews to validate the survey instrument. The questionnaires were evaluated by six faculty members and the USUHS and CDC IRBs. Three separate questionnaires were designed: (1) a pre-deployment questionnaire consisting of 4 pages, 34 questions, 47 total responses, (2) a during deployment continuation consisting of 2 pages, 17 questions, 31 total responses, and (3) a post deployment continuation consisting of 2 pages, 13 questions, and 27 total responses. The pre-deployment questionnaire was designed to

capture work, home, and hobby chemical exposures. The during and post deployment questionnaires were designed to capture perceived environmental and occupational exposures related to military deployment. After collection of the pre-deployment data, it became obvious that the exposure perception portion of the questionnaire needed an "unknown" response. Therefore, the researchers adjusted the during and post deployment questionnaire exposure perception wording to include an "unknown" response. The questionnaire collected potential exposure information and demographic information on age, sex, smoking status, occupation, and race.

2.4.2 Exposure Biomarker Collection

At the end of February 2002, the 5 person research team approached and educated potential subjects concerning participation in this study. Pre-deployment, informed consent was obtained for all 51 individuals who volunteered to participate in this research. Biologic samples (blood and urine) were collected and analyzed for chemical exposures in a total of 46 soldiers matched to themselves prior to (February 2002), during (June 2002), and after (August and September 2002) the deployment period. At pre-deployment, 51 persons volunteered for sampling. During and post deployment, the researchers were only able to obtain 48 of the 51 volunteers for follow-on sampling. After data analysis it was determined that only 46 of the original 51 volunteers completed all three phases of sampling. Therefore, the remainder of this thesis will cite 46 volunteers due to losses during sampling.

Environmental and personal dosimeter sampling were also obtained during the deployment for comparison to the exposure biomarker levels. Table 2.1 summarizes the

exact number of biological samples that were field-tested according to the methods defined in this report.

Table 2.1: Projected and Collected Number of Exposure Biomarker Samples (All Collected Pre-, During, Post)

Exposure Biomarker	**Projected Number of Samples**	**Collected Number (Pre-, During, and Post Deployment)**
VOCs in Blood	50 Subjects (150 Pre-, During, Post)	51 Subjects Pre-, 48 During, 48 Post (46 Total)
Heavy Metals in Blood and/or Urine	50 Subjects (150)	51 Subjects Pre-, 48 During, 48 Post (46 Total)
Total & Isotopic Uranium in Urine	50 Subjects (150)	51 Subjects Pre-, 48 During, 48 Post (46 Total)
Nerve Agent & Sulfur Mustard in Urine collected during deployment	50 Subjects (50 Samples Collected During Deployment)	51 Subjects Pre-, 48 During, 48 Post (46 Total)

In conducting this research, four main classes of toxic chemical exposures in deployed military personnel were evaluated. The following exposures were chosen because of their relevance to the DoD, specificity to the individual, and the readily available exposure biomarker and environmental exposure methods. They were: (1) volatile organic compounds including some of the components of JP-8 fuel, (2) total and isotopic uranium, (3) chemical warfare agents, and (4) heavy metals typically used in military paints. Exposure pathways to be addressed were: (1) inhalation, (2) ingestion, and (3) dermal absorption. All of the exposures that were evaluated are possible in the deployment environment and were evaluated concurrently with environmental monitoring of the air (inhalation), water (ingestion or inhalation), dust (dermal contact) (absorption), and soil (ingestion or inhalation).

2.4.2.1 Blood VOC Samples

A DoD phlebotomist from the deploying medical support unit collected 10 milliliters (ml) of whole blood from each of the 46 study participants (5 participants were not sampled in all three phases) pre-, during, and post deployment according to the CDC instructions for blood VOC collection (CDC, 2002). These samples were analyzed using solid-phase microextraction in conjunction with a bench top quadrupole mass spectrometer for the analysis of volatile organic compounds (VOCs) in human blood at the low parts-per-trillion level (Cardinali, 2000), the newly developed CDC method. This method is highly sensitive and specific for the following chemicals: benzene; m-xylene; p-xylene; o-xylene; ethylbenzene; toluene; methylene chloride; 1,1,1-trichloroethane; 1,4-dichlorobenzene; 2,5-dimethylfuran; carbon tetrachloride; chloroform; styrene; t-butyl methyl ether; tert-butyl alcohol; tetrachloroethene; trichloroethene. Some of these VOCs are common in military fuels and solvents. Blood samples required refrigeration and can last up to 10 weeks before degradation of the VOCs beyond analytical capability (Cardinali, 2000).

2.4.2.2 Blood Heavy Metals Samples

In addition to the 10 ml of whole blood collected above, the DoD phlebotomist collected 10 ml of whole blood from each of the 46 study participants (147 total) pre-, during, and post deployment to be analyzed using ICP-MS for heavy metals. Environmental threat assessment levels indicated that lead could have been present in the deployment environment. Therefore, blood lead samples were collected and analyzed by ICP-MS. The complete metals screen that ICP-MS can provide was reported and documented. These results are not yet available. USUHS provided funding to pay

subjects $10 per sampling event (blood specifically per regulation) given (approximately $1500 total).

2.4.2.3 Urine Heavy Metals and Chemical Warfare Agent Samples

A clean catch, 250 ml urine sample was collected from 51-study participants' pre-deployment analyzed for total uranium, isotopic uranium, and heavy metals. Two clean catch, 250 ml urine samples were collected from 48-study participants during deployment. One sample was split and frozen for shipment. This sample would be analyzed by the rapid response chemical agent screen, a newly developed CDC method and ICP-MS for heavy metals. The chemical agent screen method is capable of identifying metabolites of nerve agents and sulfur mustard. The second sample was split into two aliquots and frozen for shipment within six hours of sample collection. After shipment, AFIP analyzed samples for total uranium, isotopic uranium, and heavy metals. Post deployment, a clean catch, 250 ml urine sample was collected from 48-study participants and frozen for total uranium, isotopic uranium, and heavy metals analyses. All urine samples were required to be frozen at 0 Celsius for shipment (CDC, 1999). A total of 46-study participants provided urine at all three collection points. The chemical agent urine samples taken during deployment were held up and did not make it to the CDC before they had thawed. Therefore, chemical agent analyses were not conducted on the samples.

AFIP and USACHPPM analyzed the urine samples. Uranium analysis was specific to internal dose from the ingestion, deposition, or inhalation pathway of exposure. Uranium has a relatively short half-life in the body and therefore, it was not expected that this analysis would reveal uranium concentrations in the urine. However, use of this

method in this manner field-tested this type of sample collection and potential uses (ICRP, 1969). It should be noted that spot urine collections have been correlated to 24 hour urine collections for the kinetic phosphorescence analysis (KPA) analytical method (McDiaramid et al., 1999). It was suspected that the ICP-MS analytical method should act in the same manner, hence spot urine were collected rather than 24 hour urines (McDiaramid, 1999). The ICP-MS method is the most accurate method available today to document urine uranium levels (Bouvier-Capely etal., 2003). The ICP-MS analytical method has been validated for urine uranium measurements (Ejnik etal., 2000; Bouvier-Capely etal., 2003) and the limit of detection for the method was estimated at 0.01 parts per trillion (ppt) and 1.5 ppt for ^{235}U. The limit of quantitation varied from 3 to 5 ppt. ICP-MS affords better sensitivity and specificity and throughput than KPA or other previously used analytical techniques. The AFIP analyzed these samples and corrected for creatinine levels.

ICP-MS was also used to analyze the urine for heavy metals. Cadmium was analyzed in urine by the NHANES III study. Chromium is also best analyzed in urine (Finley, 1996). The threat assessment and analytical ease determined which heavy metals analyses were performed. Nickel, vanadium, chromium, silver, copper, manganese, and cadmium were the metals chosen to be analyzed in the urine. The laboratory provided additional metals analyses as generated in their standard analytical protocols.

2.4.3 Environmental Data

Environmental screening samples were collected in air, water, and soil for the deployment site as determined by the threat assessment. Additionally, historical and/or existing environmental chemical data were also assessed such as data from the United

Nations Environmental Programme (UNEP) and the USACHPPM. Military preventive medicine personnel were contacted to determine if any previous or on-going environmental monitoring had taken place. The pre- and post environment was not monitored.

2.4.3.1 Area Environmental Data

Environmental screening samples assisted in determining which EBs would be used during deployment. It was assumed that the chemicals listed in Table 1.2 and 1.3 would be prevalent in any deployment, but additional chemicals could have been present and guided further analysis of biological samples for those chemicals prevalent in the deployment. Environmental compliance samples in air, water, and soil for those chemicals identified during screening were collected during the deployment. Chemicals to be evaluated were VOCs, chemical warfare agents, total and isotopic uranium, and heavy metals. Environmental sampling was conducted according to EPA and OSHA methods and analyzed by USACHPPM and is reported in Appendix H. All environmental and occupational monitoring was used to calculate exposure health risks according to current methods and were compared to exposure biomarker measurements. Whenever possible, environmental laboratory analyses were completed in the same manner as the exposure biomarker analysis. However, it should be noted that these methods are costly and not commonly used in environmental sampling due to the media type (air, water, soil versus blood, urine). The methods referred to below are generally the methods that are employed for environmental analyses.

Exposure pathways to be addressed were: (1) inhalation, (2) ingestion, and (3) dermal absorption. All of the exposures evaluated were possible in the deployment

environment and were evaluated concurrently with environmental monitoring of the air (inhalation), water (ingestion or inhalation), dermal contact (absorption), and soil (ingestion or inhalation) as appropriate. The compounds selected for evaluation in this research were primarily environmental agents with accurate and practical environmental or occupational exposure monitoring methods available for their measurement. Volatile organics were monitored in the air using EPA toxic organic methods, and/or the OSHA charcoal tube method analyzed by gas chromatography (GC) with a flame ionization detector (FID), and in the water using EPA standard drinking water methods. Although the MDHEXAS did not conduct uranium environmental analyses, uranium was previously monitored in the soil of Bosnia-Herzegovina by the UNEP in the soil using a grab sample analyzed with X-ray fluorescence or ICP-MS (UNEP, 2003). The UNEP study indicated similar levels of uranium in this region (UNEP, 2003). Chemical, biological, and Radiation (CBR) warfare agents were not monitored in the air and soil because they were not expected to be present in the environment. Heavy metals were sampled in air and soil and analyzed using ICP-MS. Compounds that were analyzed using biomonitoring methods were analyzed using standard EPA and OSHA methods. The environmental or occupational exposure monitoring methods were: (1) capable of detecting exposures to an individual at a level consistent with national environmental and occupational exposure limits, and (2) relatively simple to conduct in a deployment setting. Environmental monitoring was used to conduct an environmental and occupational health risk assessment according to current methods. Risk assessment was completed using the EBs by evaluating differences between pre-, during, post deployment levels and national average. The exposure biomarker health risk assessment

and the environmental monitoring health risk assessment methodologies were compared to determine any benefits to using the exposure biomarker method. Environmental sampling was not the focus of this research, but was collected to identify any potential environmental or occupational chemical exposures to the deployed personnel, to complete an EPA risk assessment, and to investigate correlations between the blood VOC levels. Complete environmental surveillance data is detailed in Appendix H, "Deployment Environmental Surveillance Assessment, Camps McGovern and Forward Operating Base Morgan, Bosnia Herzegovina, Project No. 47-MA-7678-02" (USACHPPM, 2002).

2.4.3.2 Personal Environmental Data

Study personnel wore personal dosimeters to document levels of VOCs in the breathing zone - external dose (FMP, 2000). OVMs personal air monitoring was performed in accordance with OSHA or ACGIH Standards. Battelle shipped five day OVMs to the researchers rather than 24 hour OVMs. Therefore, Major May made the decision to use the five day OVMs as 24 hour OVMs due to the fact that the biological monitoring for Volatile Organic Compounds roughly captures the previous 24 hour exposure. The OVMs were removed from their pouches, the sampling start time and date were recorded on the reverse label, and sampler was mounted facing outward on the outside of the uniform. OVMs were worn for 24 hours (removed during showering and sleep but kept close to volunteer) and returned to the researchers at the end of the 24 hours. A brief questionnaire was completed at that time. OVMs were shipped to Battelle at the same time as the environmental samples were shipped to USACHPPM. OVMs were analyzed using gas chromatography – mass spectroscopy at Battelle Laboratories.

2.4.4 National Reference Range Data

Reference range data in the blood and urine of the general population for industrial toxins were obtained from the NHANES 1999 and NHANES III (CDC, 2001 & 2003). Currently NHANES 1999, CDC 2001, and CDC 2003 had analyzed participants for levels of 14 metals, environmental tobacco smoke, 7-8 phthalates, 8 organo-phosphates, and VOCs (CDC, 2001 & 2003). Only the reference ranges that pertain to this study were investigated. However, additional reference range data may be needed to help in refining the EBs that may be useful to DoD in the future for monitoring health risk from military relevant chemicals. Reference ranges have been published for uranium and thorium in urine of United States residents (Ting, 1999). These ranges will be considered as the common reference ranges for persons not exposed to a DoD deployment. Finally, reference ranges for heavy metal trace elements have been published for comparison (Miekeley et al., 1998). There are no available reference ranges for chemical agents in the general population because chemical agents are not naturally occurring in the environment.

2.4.5 Spatial Information

Global Positioning System (GPS) and Geographical Information System (GIS) were used to spatially orient all environmental and biological samples taken during deployment. A portable GPS instrument was used to collect spatial data. All spatial/GPS coordinates were manually recorded. This ensured that environmental samples could be modeled by their spatial patterns if a high-level existed for a specific environmental chemical. Meteorological data were also collected during deployment to include wind

direction and speed, barometric pressure, and any adverse weather such as sandstorms or rainstorms.

2.5 Data Analysis

Statistical data analyses were completed after data collection and chemical analyses were completed. Statistics included computing correlation between environmental sampling and EBs, differences between pre-, during, and post deployment levels of all toxins listed above, differences between deployment and national reference ranges (baseline) for all available toxins, and a confounder investigation for such potential confounders as smoking, age, race, and occupation. Complete data analyses are reported in the three manuscripts included in this full report.

2.5.1 Specific Aim 1 - Field test blood and urine biomarkers for use in DoD deployment missions.

First, descriptive statistics were computed for all biological chemical levels. As stated previously, geometric means were computed due to the distribution of biological data. Next, differences between pre-/during, during/post, pre-/post, highest value of pre-/during/post with the national reference ranges of toxins in the blood and urine were computed using a standard two-sided paired t-test. Paired sampling allows control of the confounding variables such as smoking, and job duties. Comparisons were not planned for the chemical agent screen because pre-, during, and post levels were not gathered. If successful, the chemical agent screen values would have been observed and group statistics reported.

Confidence intervals were used to estimate the average contaminant levels from the three groups, and inferences drawn from hypothesis testing (Student's t-test). 95% confidence intervals around the mean differences in the chemical levels in the blood and urine were computed. Frequencies of potential confounding factors were reported and used to stratify differences in blood and urine exposure levels by the confounding factors. Advanced modeling techniques were investigated to describe variability as the data allows. Table 2.2 indicates the comparisons and biostatistics that were made available to individual and group samples.

Table 2.2: Data Comparisons and Biostatistics to be Completed

Comparison	**Levels Compared**	**Descriptive & Inferential Biostatistical Test Used**
pre- to during levels	VOCs, Uranium (T&I), Metals	Two-sided paired t-test
pre- to post levels	VOCs, Uranium (T&I), Metals	Two-sided paired t-test
during to post levels	VOCs, Uranium (T&I), Metals	Two-sided paired t-test
> (pre-, during, post) to NHANES III	VOCs	Two-sided paired t-test
> (pre-, during, post) to NHANES III	Metals and Uranium	Two-sided paired t-test
pre-, during, post group averages	VOCs, Uranium (T&I), Metals	ANOVA Test
pre- to NHANES III group averages	VOCs, Metals, Uranium	Mean, Conf. Int., Student's t-test
during to NHANES III group averages	VOCs, Metals, Uranium	Mean, Conf. Int., Student's t-test
post to NHANES III group averages	VOCs, Metals, Uranium	Mean, Conf. Int., Student's t-test
Chemical Agent Levels (Failed)	Nerve agent and Sulfur mustard	Mean and Standard Deviation

2.5.2 Specific Aim 2 - Select the EBs relevant for these missions.

EB selection criteria were:

(1) Sensitive (able to detect chemical when it is present) and specific (able to indicate no chemical when chemical is not present) for common DoD chemicals/exposures,

(2) Simple (non-invasive as possible) collection method,

(3) Validated analysis method,

(4) Availability of environmental and occupational monitoring methods and standards, and

(5) Previously used to statistically determine national reference range levels.

The analytical laboratory for each test typically reports sensitivity and specificity. The ease of collection is a subjective measure that were documented through the field test trip report. Ease of analysis is another subjective measure that were documented by the laboratory completing the analysis. Finally, the availability of environmental and occupational monitoring methods and standards and national reference ranges were documented. All of these criteria were documented for each exposure biomarker used in this study and were rated subjectively. Table 2.3 indicates these criteria.

Table 2.3: Current Benefits and Limitations of Existing Environmental and Occupational Monitoring Methods

	Questionnaires, Exposure History	Environmental	Individual Air	Exposure Biomarker
External Dose	+	++	++	+
Internal Dose			+	++
Single Exposure Route (Inhalation, Ingestion, Dermal)		++	++	
Multiple Exposure Routes (Inhalation, Ingestion, Dermal)				++
Acute Exposure Assessment	+	+	+	++
Chronic Exposure Assessment	+			++
Estimation of Individual Health Risk	+		++	++
Estimation of Population Health Risk	+	++	++	+
Available for Metals	+	++		++
Available for VOCs	+	++	++	++
Available for Chemical Agents	+	++		++
Reliable		+	+	+
Sensitive		++	++	++
Specific		++	++	++
Tested in Deployments		++	+	++
Currently Used in DoD	++	+		
Opportunity for Usefulness in DoD	++	++	+	

Legend: ++ = Meets Criteria; + = Partially Meets Criteria; Blank= Does not Meet Criteria

2.5.3 Specific Aim 3 – Define correlations between EBs and environmental samples for application to health risk assessment.

Relationships between deployment VOC environmental and internal blood levels were investigated. Comparisons of during VOC levels to environmental levels (area and OVMs) were assessed using multiple linear regression analysis. The assumption was that the relationship between internal measurements (biomarkers) and external measurements (OVMs) is linear. This assumes that as the external dose increases the internal dose increases linearly. The benefits of using regression analysis are that these types of analyses allow for multiple independent variables. Other independent variables are not controlled in the relationship between external and internal dose. Therefore, regression analyses allowed control/stratification of the confounding variables such as smoking.

2.5.4 Quality Control and Data Management

Chemical data analyses results were sent to Major May from CDC, USACHPPM and AFIP. Major May analyzed and interpreted these data. All sample analysis records were kept at the analytical site (i.e. CDC, USACHPPM, or AFIP). Data analyses records and questionnaires are maintained at USUHS. USACHPPM will lock up the records without personnel identifiers in storage for a period of three years. After that time, all records will be shredded. All samples were labeled with an appropriate sequential sample number. After these samples were collected, they were stored according to analytical requirements and sent to CDC, AFIP, and USACHPPM for analysis. All samples were logged according to a sequential coding system. The code could not be traced to the individual service member by anyone but Major May. Chain of custody

forms were completed to ensure that the samples are not altered or contaminated in any way. After analyses were complete, all biological (blood and urine) samples were destroyed. Additionally, CDC, AFIP, and USACHPPM all maintain internal quality control and quality assurance measures for laboratory analysis procedures. These measures include blind and spiked analyses. Field blanks and trip blanks were collected for all air, water, soil, and OVM samples. Field and trip blanks for blood and urine were not collected for blood or urine. The CDC, AFIP, and USACHPPM quality assurance and quality control (QA/QC) measures were maintained at each respective laboratory.

CHAPTER 3: RESULTS

3.1 Results

Research results are documented in five manuscripts and one technical report. As mentioned previously, environmental data is outlined in the USACHPPM Report, Appendix H, "Deployment Environmental Surveillance Assessment, Camps McGovern and Forward Operating Base Morgan, Bosnia Herzegovina, Project No. 47-MA-7678-02" (USACHPPM, 2002). Three manuscripts are complete and two others are being written as of thesis defense. The two that have not yet been completed are the results of the heavy metals blood and urine tests and the environmental and questionnaire combined results. This is because the heavy metals blood and urine data analyses have not been completed. A sixth manuscript will be written to document the Biological Surveillance Initiative.

3.2 Manuscripts

Six peer-reviewed publications will be written to document this research. They are outlined in Table 3.1:

Table 3.1: Manuscripts

Title	Authors	Journal	Submitted	Accepted
The Recommended Role of Exposure Biomarkers for the Surveillance of Environmental and Occupational Exposures in Military Deployments	May, Weese, Ashley, Trump, Bowling, Lee	Military Medicine	Dec 2002	June 2003
Military Deployment Human Exposure Assessment: Urine Total and Isotropic Uranium	May, Heller, Kalsinsky, Ejnik, Cordero, Oberbroekling, Luong, Meakim, Cruess, Lee	Journal of Toxicology and Environmental Health Part A (JTEH A)	May 2003	July 2003
Military Deployment Human Exposure Assessment: Blood Volatile Organic Compounds (VOC)	May, Weese, Ashley, Blount, Trump, Cruess, Lee	Journal of Occupational and Environmental Medicine	Sep 2003	
Military Deployment Human Exposure Assessment: Blood and Urine Heavy Metals				
Military Deployment Human Exposure Assessment: Questionnaire & Environmental Data				
BSI Data				

Additionally, the following were written as part of this dissertation research but not as peer-reviewed publications:

1. summary report of the actual project to describe the study to study participants,
2. complete sampling plan,
3. questionnaire,
4. trip report summarizing lessons learned while applying EBs in the field,
5. exposure biomarker decision criteria, and

6. data report/fact sheet that will be mailed to all study participants to summarize and interpret the results of the research.

Title: The Recommended Role of Exposure Biomarkers for the Surveillance of Environmental and Occupational Chemical Exposures in Military Deployments: Policy Considerations

Pages: 19

Words: 3914

Tables & Figures: 5

Photos: 0

References: 15

Contact: Maj May

Guarantor: Maj May

Keywords: Biological markers, environmental exposures, risk assessment, environmental health, military personnel

Authors: Maj Lisa M. May, BSC, USAF[1]; Coleen Weese, MD, MPH[2]; CAPT David L. Ashley, PhD, USPHS[3]; CAPT David H. Trump, MC, USN[1]; Curtis M. Bowling, PE[4]; Arthur P. Lee, PhD[5]

Work Address: [1] The Uniformed Services University of the Health Sciences, Department of Preventive Medicine and Biometrics, 4301 Jones Bridge Road, Room A1044, Bethesda, Maryland 20814-4799; [2] The US Army Center for Health Promotion and Preventive Medicine, Occupational Medicine, 5158 Blackhawk Rd., Aberdeen Proving Ground, Maryland 21010-5403; [3] The US Centers for Disease Control and Prevention, National Center for Environmental Health, 4770 Buford Highway, N.E., Mailstop F-47, Atlanta, Georgia 30341-3725; [4] The Assistant Deputy Under Secretary of Defense (Safety and Occupational Health), The Pentagon, Room 3E791, Washington, District of Columbia, 20301-3400; [5] The US Army Center for Health Promotion and Preventive Medicine, Deployment Environmental Surveillance Program, 5158 Blackhawk Rd., Aberdeen Proving Ground, Maryland 21010-5403.

Previous Presentations: None

Citation: May, LM et al. "The role of exposure biomarkers for the surveillance of environmental and occupational chemical exposures in military deployments: policy considerations". *Military Medicine* 169(10) 761-767.

Note: The above article is not included due to copyright restrictions. Pages 63-86 have been deleted.

Journal of Toxicology and Environmental Health Part A (JTEH A): TITLE PAGE

Title: Military Deployment Human Exposure Assessment: Urine Total and Isotopic Uranium Sampling Results

Authors: Maj Lisa M. May, BSC, USAF[1]; Jack Heller, PhD[2]; Victor Kalasinsky, PhD[3]; John Ejnik, PhD[3]; Steve Cordero, MS[3]; Kristi J. Oberbroekling[3]; Thuy T. Luong[3]; Kathryne C.E. Meakim[3]; David Cruess, PhD[1]; Arthur P. Lee, PhD[2]

Work Address: [1] The Uniformed Services University of the Health Sciences, Department of Preventive Medicine and Biometrics, 4301 Jones Bridge Road, Room A1044, Bethesda, Maryland 20814-4799; [2] The US Army Center for Health Promotion and Preventive Medicine, Deployment Environmental Surveillance Program, 5158 Blackhawk Rd., Aberdeen Proving Ground, Maryland 21010-5403; [3] The US Armed Forces Institute of Pathology, Environmental and Toxicology Chemistry, 6825 16th St. N.W., Washington, District of Columbia 20306-6000.

Keywords: Exposure Biomarkers, Environmental Chemicals, Military Deployments, Uranium, Urine Sampling

Citation: May, LM et al. "Military Deployment Human Exposure Assessment: Urine Total and Isotopic Uranium Sampling Results". *Journal of Toxicology and Environmental Health Part A* 67(8-10): 697-714.

Journal of Occupational and Environmental Medicine: TITLE PAGE

Title: Military Deployment Human Exposure Assessment: Blood Volatile Organic Compound Sampling Results

Authors: Maj Lisa M. May, BSC, USAF[1]; Jack Heller, PhD[2]; David L. Ashley, PhD[3]; Benjamin C. Blount, PhD[3]; CAPT David H. Trump, MC, USN[1]; LTC Michael Roy, MC, USA[1]; Coleen Weese, MD, MPH[2]; David Cruess, PhD[1]; Arthur P. Lee, PhD[2]

Work Address: [1] The Uniformed Services University of the Health Sciences, Department of Preventive Medicine and Biometrics, 4301 Jones Bridge Road, Room A1044, Bethesda, Maryland 20814-4799; [2] The US Army Center for Health Promotion and Preventive Medicine, Deployment Environmental Surveillance Program, 5158 Blackhawk Rd., Aberdeen Proving Ground, Maryland 21010-5403; [3] The US Centers for Disease Control and Prevention, National Center for Environmental Health, 4770 Buford Hwy, N.E., Building 103, Atlanta, Georgia 30341-3725.

Keywords: Exposure Biomarkers, Biological Markers, Environmental Exposures, Environmental Chemicals, Military Deployments, Volatile Organic Compounds, Air Sampling

ABSTRACT:

Currently the Department of Defense (DoD) does not use exposure biomarkers to measure Service Members' exposure to environmental chemicals. Blood and urine exposure biomarkers for volatile organic compounds (VOC), selected heavy metals, depleted uranium (DU), and chemical warfare agents are currently available but have not been field tested or validated by the DoD in military deployments as a tool to document exposures. The Military Deployment Human Exposure Assessment Study, a prospective cohort of 46 soldiers deployed to Bosnia, was designed to field test blood and urine exposure biomarkers as a mechanism to document exposures to these chemicals during military deployments. Blood and urine were collected pre-, during, and post-deployment. Standard questionnaire, environmental and occupational monitoring methods were conducted for comparison to the exposure biomarker results. This paper compares and describes the pre-, during, and post-deployment blood VOC results, compares these to standard US blood VOC levels, reports deployment environmental and occupational measurements, and attempts to correlate environmental with blood VOC results. VOCs were detectable but below the national reference ranges except in the case of styrene. VOCs measured in human blood did not correlate well with individual environmental samples. Finally, study outcomes indicate that questionnaire data and standard environmental data are not adequate to evaluate DoD exposures and risks from environmental and occupational chemicals.

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INTRODUCTION:

The difficulty of collecting individual exposure information hampered the evaluation of exposure-outcome relationships following the 1990-91 Gulf War. Exposure concerns during Operation Enduring Freedom and Operation Iraqi Freedom deployments have amplified interest in individual environmental and occupational chemical exposure assessment. Currently, deployment assessments are conducted using intermittent ambient air monitoring, occasional focused evaluations based on these results, and post-deployment questionnaire documentation of exposure and/or health concerns. While this strategy is an improvement over prior practices, it has limitations including a reliance on evidence of an acute problem to initiate or provide in depth individual health evaluations. Exposure biomarkers may have the potential to overcome some of the limitations of current environmental and occupational exposure assessment tools. Exposure biomarkers have not been field tested for use in Department of Defense (DoD) deployments as an exposure assessment tool. The Military Deployment Human Exposure Assessment Study (MDHEXAS) field tested blood and urine exposure biomarkers for volatile organic compounds (VOCs), uranium, heavy metals, and chemical agents in these scenarios. Two previous publications currently "in press" document the military and overall public health implications of these methods (May, 2003a). This paper reports the results of blood VOC exposure biomarker field test. An earlier paper reports the results of the urine uranium exposure biomarkers and the subsequent papers will report the results of the blood and urine heavy metals exposure biomarkers, and the complete environmental and questionnaire data (May, 2003b).

Military and Public Health Significance

Exposure Biomarkers (EBs) have been recommended for use in DoD by three documents. These are: (1) the 1 February 2002 Joint Chiefs of Staff Memorandum titled, “Updated Procedures for Deployment Health Surveillance and Readiness”; (2) the August 1998 Presidential Review Directive 5 titled “Planning for Health Preparedness for and Readjustment of the Military, Veterans, and Their Families after Future Deployments”; and (3) the 2000 Institute of Medicine (IOM) Report, “Protecting Those Who Serve”. However, exposure biomarkers have not been field tested as a human exposure assessment methodology for use in DoD.

In January 2000, the Uniformed Services University of the Health Sciences (USUHS) proposed a collaborative prospective epidemiological research study to field test exposure biomarkers for military relevant chemicals during deployments. This study titled, “The Military Deployment Human Exposure Assessment Study (MDHEXAS)” was designed to survey the military population and serve as companion to the Environmental Protection Agency’s, “National Human Exposure Assessment Survey (NHEXAS) Study (Robertson et. al., 1999). Additionally, the MDHEXAS was designed with the vision that once completed, the US Centers for Disease Control and Prevention (CDC) would have released their National Report and Second National Report on Human Exposure to Environmental Chemicals in early 2001 and early 2003 (CDC, 2001; CDC, 2003). The MDHEXAS was completed in September 2002 and this paper serves as the second report of the study results. The MDHEXAS research objectives were to (1) field test blood and urine exposure biomarker methods in a prospective DoD deployment

scenario, (2) select the exposure biomarkers best suited for militarily relevant toxic chemicals, and (3) determine correlations between exposure biomarkers and traditional environmental samples (area and personal). The importance of this research is that it fills the gap for a scientifically tested internal dose measurement method for exposures during DoD deployment activities. This paper will summarize the results of the MDHEXAS specific to VOC exposures.

VOCs, components of fuels and paints, are used for various military applications including refueling, painting, and degreasing. Therefore, exposure may be relatively common and overexposure, particularly in areas with limited ventilation, can cause health impacts such as headaches, nausea, and dizziness. There have been periodic reports of such overexposures in deployed settings, where typical engineering controls are not necessarily in place. Although ambient air analysis includes VOCs, personal and area sampling for VOCs is not conducted. Therefore, measurement techniques are required to accurately preclude or include and document population and/or individual human exposures to VOCs.

BACKGROUND:

Exposure biomarkers were selected for study in the MDHEXAS if the following criteria were met. These were:

(1) The exposure biomarker must be both sensitive (able to detect chemical when it is present), specific (able to distinguish target chemical from potential interferences) and must be able to provide internal dose estimates.

(2) The exposure biomarker must have a simple (less invasive) collection method

that does not put the individual at risk.

(3) The analytical method must be validated and biological variability should be low.

(4) Environmental and occupational monitoring methods and standards relevant to the exposure biomarker must be available. The quantitative measurement capability of the exposure biomarker should be within the range of the measured environmental exposure which allows adequate statistical comparisons and correlations between internal (i.e. blood) and external (i.e. air) measurements.

(5) The exposure biomarker should have known national reference range levels for non-occupationally exposed members of the general population to aid in the interpretation of measured exposures. Currently, the National Health and Nutrition Examination Survey conducted by CDC provides national reference range levels for selected environmental chemical hazards (CDC, 2001; CDC, 2003).

(6) The exposure biomarker concentrations measured should quantitatively relate to health effect, or have some prognostic value. The toxicokinetics or persistence of the chemical in blood or urine following exposure must be known.

(7) Finally, the exposure biomarker should provide useful information over and above that obtained by ambient monitoring (Ashford, 1990).

Specific to VOC exposures, the purpose of the MDHEXAS was to document environmental and individual VOC exposure. Therefore, in addition to questionnaire and environmental data specific to VOCs, individual environmental and biological monitoring was conducted. This was accomplished with individual environmenal air monitoring conducted using Battelle's newly designed deployment 24 hour Individual

Passive Chemical Samplers (IPCS) that measure both organic vapors and chemical agents (USASBCCOM, 2002). Since this report documents VOC exposures, the passive samples used will be referred to as Organic Vapor Monitors (OVMs). It is important to note that individual environmental sampling was not conducted for heavy metals or uranium because these methods are still extremely difficult to deploy in military operations. The OVM data allow correlations between environmental air levels and human blood levels to be examined and described for the first time in military deployments. In the MDHEXAS, blood samples were taken immediately following the 24 hour OVM sample period thus meeting the time restrictions.

Accumulation of chemicals in the body occdurs whenever uptake exceeds elimination. The studies done to determine VOC pharmacokinetics also suggest that with repeat exposure of long enough duration, bioaccumulation may occur. Some measurements have been performed on workers repeatedly exposed to VOCs over a matter of weeks. Berlin et. al. exposed volunteers to low levels of benzene over 5 days for 6 hours per day (**Berlin et. al,**). These workers showed accumulation during the exposure period and continued to release benzene for more than a week after the exposure ended. Brugnone et. al. found bioaccumulation of styrene in workers exposed repeatedly over a week (**Brugnone et. al.,**). Nise and Orbeck found this same result in workers who were repeatedly exposed to toluene (**Nise and Orbeck,**). Preshift levels of these VOCs in workers increased during the week they were exposed because their internal dose levels had not returned to baseline between exposures. Bioaccumulation in VOC exposure is important because most exposures to these compounds occur repeatedly and are usually not one-time events. Thus, although short-term exposure experiments

give insight into the pharmacokinetics of VOCs, they are of limited value in most exposure scenarios. In repeat exposure cases, the exponential component with the longest half-life will have the greatest influence on internal dose levels, and in many cases bioaccumuloation can occur. The extent of bioaccumulation will depend on the level of exposure, the length of time during which exposure occurs, and the time period between exposure events (Ashley et. al., 1996). Additionally, physiological characteristics such as gender, age, body mass index, and the effect of exercise all contribute to variation in internal VOC dose (Klassen, 2001).

For this research, it is expected that personnel would be protected against high-level VOC exposures (personal protective equipment or engineering controls). However, during deployments it is possible that personnel are being exposed to low-level VOCs and monitoring and analysis of their blood is warranted. Table 1 lists the metabolic pathways for the VOCs under study. It should be noted that these metabolic pathways are primarily descriptions of occupational exposures to VOCs. The occupational data are being used to describe degradation because it is the best studied evidence of degradation that exists at this time. The parent VOC will be sampled and analyzed in the blood because of expected low-level deployment environmental exposures. As is evident in the table, VOCs metabolize to their degradation products at varying, relatively quick rates. In the body, there are many pathways to form VOC metabolites. Additionally, VOC metabolites are found at low levels in the majority of humans thus causing difficulty in determining background levels of a metabolite in low-level environmentally exposed persons such as this study. VOC metabolites are not specific to the compounds of toxicological concern. The parent compound can also degrade at varying degrees but the

laboratory results are more specific to the compound due to the analytical instrumentation. The MDHEXAS attempted to document VOC in blood of individuals within 24 hours of exposure through the collection and analysis methods for VOC blood and OVM samples.

METHODS: Blood samples were collected and analyzed using solid-phase microextraction in conjunction with a bench top quadrupole mass spectrometer for the analysis of volatile organic compounds (VOCs) in human blood at the low parts-per-trillion level (Cardinali, 2000), a newly developed CDC method. This method is highly sensitive and specific for thirty one VOCs including the following: benzene; m-/p-xylene; o-xylene; ethylbenzene; toluene; methylene chloride; 1,1,1-trichloroethane; 1,4-dichlorobenzene; 2,5-dimethylfuran; carbon tetrachloride; chloroform; styrene; t-butyl methyl ether; t-butyl methyl ether; tetrachloroethene; trichloroethene. Data collection pre-, during, and post-deployment was completed on 13 September 2002. To determine a difference between pre-, post-, during, and national reference range VOC levels in the blood, 34 persons were required for sampling (effect size 0.5, alpha of 0.05, beta of 0.05 for a standard two-sided t-test) (Cohen, 1998). Fifty persons were required per each set of blood and urine collected to account for an expected 20% loss to follow up at each stage of sampling.

Study Cohort

Prior to approaching potential study subjects, full Institutional Review Board (IRB) reviews and approvals were obtained from USUHS and CDC. Soldiers deployed to Bosnia participated in this study and provided blood and urine samples for analysis

according to the exposure biomarker protocol described herein. Environmental and individual environmental (OVM) sampling was also obtained during the deployment for comparison to the exposure biomarker levels. The experimental design for this research was a prospective, methodological cohort. Entry criteria were: (1) unit with greater than 50 persons, (2) age ranging from 18 to 55, (3) either male or female, and (4) serving in “active duty” military status. There were no exit criteria. Volunteers were enrolled in the study if they provided informed consent to participate and were deploying on “active duty” military status. It is important to note that neither the location nor cohort of individuals was selected due to expected exposures or over-exposures to environmental chemicals. The cohort was selected simply because of availability of members to volunteer for the study. The follow-up period was the duration of the deployment, approximately 6 months. After informed consent was obtained, study subjects provided blood and urine samples and completed a pre-deployment exposure biomarker questionnaire. Study subjects were processed through the MDHEXAS with strict quality control techniques so that all informed consent was obtained, and questions were carefully examined. Biologic samples (blood and urine) were collected and analyzed for chemical exposures in a total of 46 soldiers matched to themselves prior to (February 2002), during (June 2002), and after (August and September 2002) the deployment period. At pre-deployment, 51 persons volunteered for sampling. During and post-deployment, the researchers were only able to obtain 48 of the 51 volunteers for follow-on sampling. After investigation, only 46 of the original 51 volunteers completed all three phases of sampling. Therefore, the remainder of this manuscript will cite 46 volunteers due to losses during sampling. It is not expected that those volunteers leaving

the study would bias the findings of this study in any way. Volunteers were free to extricate themselves from the study at any time. The 5 persons who did not give blood during all 3 phases of collection did not extricate from the study. Three of the personnel were not physically located on Camp McGovern during the 2-15 Jun 03 sampling period and therefore could not provide a blood sample. The remaining two did not return to Ft Dix during the times that the research team was collecting samples and likewise could not provide blood for the study.

To determine over-exposure to toxic chemicals in blood and urine, the CDC National Health and Nutrition Examination Survey (NHANES) cohort data was used as the external referent comparison group for this study. The non-occupationally exposed reference sample of the NHANES cohort provided context for VOC levels in blood found in this study. These referent data are not reported in the CDC National Report or second National Report on Human Exposure to Environmental Chemicals (CDC, 2001; CDC, 2003). In the next release of this report scheduled for 2005, the NHANES cohort will be evaluated using blood VOC measurement techniques.

Questionnaire Design

The questionnaire was designed for two purposes: (1) to collect demographic data, and (2) to document environmental exposure perception. Questionnaire design was conducted using standard survey design techniques and included a set of cognitive interviews to validate the survey instrument. The cognitive interviews were conducted at Aberdeen Proving Ground, Maryland in October 2001. The questionnaires were evaluated by six faculty members and the USUHS and CDC IRBs. Three separate questionnaires were designed: (1) a pre-deployment questionnaire consisting of 4 pages,

34 questions, 47 total responses, (2) a during deployment continuation consisting of 2 pages, 17 questions, 31 total responses, and (3) a post-deployment continuation consisting of 2 pages, 13 questions, and 27 total responses. The pre-deployment questionnaire was designed to capture work, home, and hobby chemical exposures. During and post-deployment questionnaires were designed to capture perceived environmental and occupational exposures related to military deployment. After collection of the pre-deployment data, it became obvious that the exposure perception portion of the questionnaire needed an "unknown" response. Therefore, the researchers adjusted during and post-deployment questionnaire exposure perception wording to include an "unknown" response.

Environmental Sampling

There are several methods available to document area and individual environmental exposures but none are entirely conducive to the deployment environment. Area environmental samples consist of ambient air, water, and soil sampling. Complete environmental sampling and analysis will be described in subsequent manuscripts and is documented in the USACHPPM Deployment Environmental Assessment, Camps McGovern and Forward Operating Base Morgan, Project Number 47-MA-7678-02 (USACHPPM, 2002). All 46 persons assessed using biological sampling techniques were assigned to Camp McGovern and therefore, the environmental data reported do not include Forward Operating Base Morgan. Camp McGovern is located in the northern part of Bosnia Herzegovina near the Croatian boarder outside the town of Brcko. The Camp was establish in 1996 and houses U.S. military and contractor personnel. The camp

consists of multiple permanent and semi-permanent buildings connected with gravel roads/paths (USACHPPM, 2002).

VOC's were collected using two modified Environmental Protection Agency (EPA) methods, Toxic Organic 17 (TO-17) and EPA TO-14. Modified TO17 method consists of using a carbo-trap triple-bed sorbent tube and a low volume personal sampling pump to collect an area sample over an 8-hour (480 minute) period. The sampling rate on the pump was set to approximately 40 mL/min to collect a total sample volume of approximately 20 liters. Contaminants were absorbed on the sample media during the sampling period. For each sample period, a primary and co-located sample were collected and submitted with a field blank to the laboratory for analysis. The practice of collocating samples and submitting samples with field blanks were done to insure quality control and quality assurance (QA/QC). These samples were analyzed to determine VOC concentration of contaminants in ambient air using a gas chromatograph/mass spectrometer (GC/MS).

TO-14 method uses a 6-liter stainless steel, silica-lined canister that is cleaned and evacuated to negative 30 inches of mercury. A sample is passively collected over a 24-hour period using a flow restriction device. These samples were analyzed to determine the concentration of contaminants in ambient air using a GC/MS (USACHPPM, 2002).

Two ambient air-sampling sites were established at Camp McGovern, at the center of the camp in life support area (LSA) and at the southwest corner of the camp next to the maintenance facility and refueling point. Each site consisted of two Mini-Vol samples, one set of modified TO-17 samplers (two sampling pumps and two sorbant tubes) and one TO-14 canister (EPA, 1983). A Mini-Vol sampler is a pump designed to pull air

through a filter or cartridge. If the air contains environmental chemicals, the filter or cartridge can be shipped to an analytical laboratory and analyzed. Mini-Vol and TO-14 canisters were used to collect 24-hour composite samples (USACHPPM, 2002). Modified TO-17 samplers were used to collect 8-hour composite samples. In addition ambient temperature, ambient pressure, and site conditions were recorded during the sampling.

Bottled water was the primary drinking water at Camp McGovern during the time of this study. At the time of the Survey, Fonte Guizza bottled water from Italy was being used. Bottled water supplier varies depending on current contractual requirements and Veterinary Command (VETCOM) approval of sources. Camp McGovern also contains a water treatment plant operated by Kellog, Brown, and Root Services. The plant draws water from two wells on the camp and treats the water using sand filters, micron filters, ultraviolet disinfection and chlorination. Water is then distributed to the Wagon Wheel Dining Facility (DFAC) and other food handling facilities for dish and hand washing. It is also distributed to the Camp's semi-permanent latrine and showering facilities (USACHPPM, 2002).

Both bottled and tap water was sampled by the research team to completely describe all sources of exposure. Tap water was not a primary drinking water source at Camp McGovern however could have been used as drinking and/or cooking. Potable water samples were collected from the tap at the DFAC, well #1 and well #2. In addition, three 500 milliliter bottles of Finte Guizza water were collected for analysis. Water samples were collected at Camp McGovern using the deployment potable water sampling kit. The kit was designed to test treated water and groundwater sources for military

deployments (USACHPPM, 2002). Water samples taken with the deployment water sampling kit are analyzed for heavy metals, organophosphates, VOCs, pH, hardness, and other standard water sampling methods.

The majority of Camp McGovern is covered with gravel, with limited paved areas. The paved areas are mainly in the helipad area and parking area. There is very little vegetation (e.g. trees, shrubs and bushes) on the camp, however there are several grassy areas; these are mainly at the softball and soccer field areas. Three soil samples were collected at Camp McGovern.

Individual environmental samples were obtained through personal dosimetry of VOCs on OVMs which are passive sampling devices, sometimes referred to as badges that are commonly worn by workers to document exposures over time. Industrial hygiene air sampling pumps can also be used to collect air in the breathing zone of a worker onto a filter that can be analyzed for various chemicals including a suite of VOCs.

OVM exposure badges were used in the MDHEXAS for VOCs because they are inexpensive, easy to use, and validated for exposure assessment. Study personnel wore personal dosimeters to document levels of VOCs in the breathing zone - external dose (FMP, 2000). OVM monitoring was performed in accordance with OSHA or ACGIH Standards. OVMs designed to capture five day VOC exposure were used to document 24 hour VOC exposures due to the fact that: (1) these were available for immediate testing, and (2) the biological monitoring for VOCs roughly captures the previous 24 hour exposure. OVMs were analyzed using thermal desertion transferred to gas chromatography at Battelle Laboratories (USASBCCOM, 2002).

Biological Sampling

VOCs are exhaled and/or metabolized relatively rapidly; therefore, samples should be obtained either before the individual is removed from exposure or as quickly after exposure as possible (CDC, 1999). The blood VOC samples collected during the MDHEXAS were focused on chronic environmental exposures. Therefore, samples were collected mid-deployment to Bosnia not after a known acute exposure period such as a work shift following use of a specific chemical. The assumption was that chronic environmental levels would have stabilized in the blood of the individual after living in the Bosnian environment for three months. Whether the samples are aimed at documenting chronic or acute exposures does not impact the need for special sample collection considerations.

Due to potential VOC contamination, sampling materials must be handled carefully. Vacutainer tubes obtained from commercial sources contain VOC contamination which can greatly interfere with the ability to obtain analytical results which are indicative of the degree of exposure. To avoid this interference, the sample tubes used in the MDHEXAS were obtained commercially and specially modified at CDC so that they no longer contained measurable levels of most VOCs. The anticoagulant used in the CDC prepared tubes was a mixture of potassium oxalate and sodium fluoride. This anticoagulant is chiefly intended to stop metabolism so that VOC levels do not change appreciably during storage (CDC, 1999). Once samples were collected, they were mixed thoroughly to allow the complete distribution of the anticoagulant (CDC, 1999).

Isopropanol used to disinfect the venipuncture site has resulted in interferences in the analytical measurement by introduction of this compound into samples. Isopropanol

contamination was minimized by swabbing the site with a dry gauze bandage and allowing the site to dry for 5 - 10 seconds after wiping with isopropanol (CDC, 1999). All samples were placed on wet ice or into a refrigerator within 30 minutes of sample collection to avoid degradation of VOCs. Samples were shipped with enough wet ice or equivalent cooling material to insure that the samples remained cool throughout the shipment process.

Solid-phase microextraction coupled with gas chromatography and mass spectroscopy (SPME-GCMS) was used to accurately quantify blood VOC levels (Cardinali, 2000). Detection limits varied by analyte, ranging from 0.005 ng/mL for 1,1 dichloroethane, carbon tetrachloride, and dibromochlomethane to 0.12 ng/mL for 1,4-dichlorobenzene (CDC, 2002). SPME-GCMS affords better reliability and throughput than previously used analytical techniques such as purge and trap GC-MS (Ashley, 1996). The CDC analyzed these samples in accordance with their protocols and quality control program.

Three statistical comparisons were conducted using the blood VOC data. First, the pre-, during, and post-deployment levels for each of the 46 study volunteers were compared by a paired t-test with an alpha set at 0.05 and using the Bonferronni correction to adjust for multiple comparisons. Pre-, during, and post-deployment blood levels were not compared statistically against national reference data because data did not differ significantly. The McNemar Chi-Square test was computed for incomplete pairs of blood and OVM data during deployment. Finally, linear regression was conducted between blood and OVM data for ethylbenzene, xylene, styrene, and toluene. These compounds

had probability distributions that were normal thus meeting the assumptions of linear regression. In the case of regression analysis, if a data point was missing then one half the detection limit was used to replace those samples that were not detected during analysis. Too many non-detected results would skew the distribution and therefore, this method was not used when over half the data points were non-detect.

RESULTS:

Questionnaire

Forty-six military members (43 men, 3 women) had complete pre-, during, and post-deployment assessments (Table 2). The prevalence of smoking was reported on the questionnaire as 67% pre-deployment and post-deployment, but increased to 74% during the deployment. Table 3 displays their self-reported exposures to passive smoke, fuels, paints, solvents, and chemical warfare agents. Smoking is a potential source of VOCs and metals in blood therefore, exposure to tobacco smoke was confirmed by quantifying blood levels of 2,5-dimethylfuran (Ashley et. al., 1996). Self-reported perception of working with chemicals decreased from 37% pre-deployment to 22% both during and post-deployment. Pre-deployment, 57% of the cohort perceived exposure to chemical warfare agents while during and post-deployment respectively 39% and 46% with 20% of the cohort responded “unknown” to the question regarding their perception of chemical warfare agents exposure. The pre-deployment questionnaire did not provide the opportunity to give “unknown” as a response; thus it is not clear what fraction of those reporting perceived exposure would have selected unknown. Exposure to chemical

warfare agents did not occur during the deployment as assessed by environmental and biological monitoring; underscoring the value of this information to a population concerned about potential health consequences of perceived exposure.

Environmental Sampling

Table 4 documents the ambient air VOC levels in Bosnia during the time period of biological monitoring (USACHPPM, 2002). VOCs were not detected in the soil at Camp McGovern, Bosnia (USACHPPM, 2002). VOCs were only detected in three water samples by EPA 524.2 analytical method. They were bromodichlormethane at 0.001 mg/L, chloroform at 0.0012 mg/L, and dibromochloromethane at 0.0007 mg/L. The detection limit for all three samples was 0.0005 mg/L. As expected, VOCs volatilize rapidly in water and therefore are not thought to be the primary route of exposure. The complete data are documented in the USACHPMM reference, Deployment Environmental Assessment, Camps McGovern and Forward Operating Base Morgan, Project Number 47-MA-7678-02 (USACHPPM, 2002).

Table 5 documents the OVM averages for the 46 persons matched to blood analyses. Half of the detection limit was substituted for those values reported below the detection limit. This is a more conservative approach than assuming zero exposure. No chemical agents were detected on the IPCSs used during this study (USASBCCOM, 2002).

Individual Blood Sampling and Comparison of Pre-, During, Post- VOCs

Tables 6-12 document geometric mean, confidence interval, and range of blood benzene, toluene, ethylbenzene, xylene, styrene, and 2,5-dimethylfuran levels pre-, during, and post-deployment to Bosnia. The average (geometric mean) blood VOC levels were calculated using half the detection limit for those values reported below the

detection limit. Geometric means were reported only for those compounds which were detected more than 5 times pre-, during, and post-deployment. Total xylene was calculated by combining m-/o-/p-xylene primarily because the OVM averages reported did not match to the blood analyses.

To understand whether the values of blood VOCs were significantly different during deployment, we conducted a paired t-test. Tables 6-12 also depict the results of the paired t-test for three comparisons: (1) pre-deployment to during deployment, (2) during deployment to post-deployment, and (3) pre-deployment to post-deployment. The following were statistically significantly different:

1. Pre- to During and Pre- to Post- levels of ethylbenzene,
2. Pre- to Post- levels of xylene,
3. Pre- to During, Pre- to Post-, and During to Post- levels of styrene, and
4. During to Post- levels of toluene.

It is critically important to note that although statistically different, the results of blood VOC analyses were so low (e.g. 0.01 parts per trillion) that the realistic difference in exposure may be negligible.

Comparison of Blood VOC Deployment Results to US Standard Reference Ranges

Tables 6-12 also depict the US Standard Reference Ranges of the VOCs that were detected in the blood of the Task Force 1-151 volunteers as reported by the CDC from the NHANES III reference study (Churchill et. al., 2001). In the MDHEXAS, the geometric mean and confidence intervals only approach the national reference range for styrene post-deployment. The styrene post-deployment geometric mean was 0.14 ng/mL and the national reference range geometric mean is 0.074 ng/mL. Additionally, styrene

exposures are significantly different pre- to during and post-deployment. The styrene data suggest that exposures were more than “normal” as compared to the general US population (CDC, 2003). However, the NHANES referent group may not be entirely appropriate to a military cohort. It would be beneficial to investigate the need for a military NHANES cohort as a stronger external comparison group.

Correlation of Individual Air and Blood Sampling

To determine how well air sampling predicts the blood levels, individual air sampling measurements were correlated to the blood VOC measurements for the deployment study period. Comparisons could only be computed between those contaminants that were analyzed and detected in greater than 20% of both the OVM samples and the blood. Table 8 outlines the non-parametric analyses and the linear regression analyses.

DISCUSSION:

There is no evidence that members of this cohort were subject to significant environmental VOC exposures as measured by questionnaire, environmental analyses, OVM, and/or blood analyses. The MDHEXAS shows the importance of all aspects of a comprehensive environmental surveillance program to include individual exposure monitoring as obtained through exposure biomarkers. Environmental and biological measurements confirm that the cohort was exposed to both passive smoke and fuels. Conversely, neither exposure assessment method identified any exposure to chemical warfare agents. This research supports the complementary nature of using both environmental data and individual biological data for determining exposure.

Exposure biomarkers offer individual measurements of exposure that are more difficult to dispute than indirect measurements of exposure. However their interpretation may pose a controversy. The assumptions of exposure are either confirmed or not confirmed through the capture of a biological sample.

Smoking status increased during deployment from 67% to 74% of the cohort. Post-deployment smoking returned to 67% of the cohort. Exposure to tobacco smoke was confirmed by quantifying blood levels of 2,5-dimethylfuran. Blood levels of 2,5-dimethylfuran reflect tobacco smoke exposure over the last few hours, whereas serum or urinary continine integrates exposure over a longer time period. Therefore, a light smoker who has not smoked in the last 3-4 hours may not have measurable levels of 2,5-dimethylfuran. Tobacco smoke contains many volatile organic compounds and thus smoking status is useful for interpreting VOC results. In the Ashley, 1996 study, 2,5-dimethylfuran was reported as 96% predictiveof smoking status. Exposure to tobacco smoke is asessed by quantifying blood levels of 2,5-dimethylfuran, with detectable levels (>0.011 ng/mL) indicating exposure (Ashley et. al., 1996). The 2,5-dimethylfuran data indicated that 45% (20/44) of the study participants were non-smokers with minimal exposure to second hand smoke. Whereas, the self-reported questionnaire data indicate between 64-74% of volunteers were smokers. The overall magnitdue of exposure to tobacco smoke increased during deployment, indicative of increased smoking by some study participants. This may partially explain the increased levels of volative aromatic hydrocarbon comounds: benzene, toluene, ethylbenzene, ortho-xylene, meta-/para-xylene (“BTEX”), and styrene when comparing pre- and during deployment levels.

The only compound above national reference ranges was styrene. The

measurements taken during the MDHEXAS suggest that Bosnia and/or deployment styrene exposures may be elevated. Increases in styrene can be explained due to an increase in tobacco smoking or increased environmental levels of styrene in Bosnia's air, food, soil, or water. Most VOCs enter the body by inhalation and therefore, the increase in blood styrene levels were possibly due to increased cigarette smoking. Food was not monitored for environmental contaminants due to the technical difficulties associated with food sampling and analysis.

Although many of the cohort members perceived exposures to chemical agents, often neither blood nor OVM measurements supported this perception. This fact can be documented in an individual's medical record for future evaluation and medical follow up. Interpretation of the exposure biomarker results is not completely documented in the scientific or government regulatory communities largely because the exposure biomarker techique is relatively new to environmental epidemiology. As these methods become more available and further validated, we expect to see two outcomes. The first will be the creation of standards or methods of interpreting the results of blood and urine samples documenting exposure to environmental chemicals. The second will be the influx of more accurate environmental epidemiology studies due to better exposure documentation techniques. Currently, environmental epidemiologists rely largely on questionnaire data to document exposure.

As in any sampling method, uncertainty and sources of error exist. The most significant source of error in the exposure biomarker method is the time of sampling. It is entirely possible to miss exposures completely due to the clearing mechanisms (exhalation/toxicant metabolism) of the individual. In the case of VOCs, this is

particularly concerning and disruptive of individual exposure documentation accuracy and validity. Therefore, continued validation studies are necessary to better describe the effects of human metabolism. Other sources of uncertainty include both inter- and intra-person variability. Both sources of variabilty can be managed by increasing the sample size of the cohort studied (military units) and the reference population (NHANES). The current CDC data base is adequate to minimize the effects of intra-person variability in the control population. However, the CDC NHANES data do not account specifically for military populations. Therefore, a military NHANES would be a valuable investment for the DoD prior to additional implementation of biological monitoring. Individual variability can be managed with increasing sampling events and ensuring that samplings include each person as their own control such as in the pre-, during, post- model of the MDHEXAS.

CONCLUSIONS and RECOMMENDATIONS:

In closing, it is recommended that biomarkers be deployed in conjunction with standard occupational and environmental monitoring methods such as was done in the MDHEXAS. This allows for more accurate health risk assessment validation. The results of the field test indicate that exposure biomarkers may be a valuable tool to the DoD in exposure and risk assessment from environmental and occupational chemicals. In the MDHEXAS, exposure biomarkers indicate increased levels of styrene.

The geometric mean of blood VOC levels were not above U.S. national reference ranges except in the case of styrene which was only slightly above the national reference range. Therefore, adverse health effects are not expected to be different from US levels

on a population basis due to VOC exposures to the Task Force 1-151 Indiana National Guardsman who deployed to Bosnia March – September 2002 in support of Stabilization Force. Blood VOC analyses appear to provide specific data for individuals that indicate increased exposure to certain environmental VOCs during deployment. The levels of blood VOCs although different during and post-deployment remained in the "normal" range.

Finally, exposure biomarkers are not generally recommended for screening. Especially in the case of the blood VOC exposure biomarker where physiological kinetics influence the results of the test greatly. It is therefore recommended that specific evaluations of exposure linked to job types or activities or suspected environmental exposures be the focus of blood VOC exposue biomarker evaluations.

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Table 1: VOC Metabolites (Occupational Settings)

VOC	Metabolite	Recommended Time Limit – Post Exposure	Biological Exposure Indices
Benzene	Trans, trans-muconic acid, phenyl mercapturate, phenol	15 hours	50 mg/g creatinine (urine)
Toluene	o-cresol, benzyalcohol, benzaldehyde, benzoic & hippuric acid	8 hours	1 mg/L (blood)
Xylene	methylbenzlalcohol, dimethylphenol, methybenzoic acid and methylhippuric acid	16 hours	1.5 g/g creatinine (urine)
Ethylbenzene	1-phenylethanol, acetophenone, hydroxyacetophenones, and phenylglyoxylic acid		1.5 g/g creatinine (urine)
Methylene Chloride	by P450 cytochrome to carbon monoxide, and in conjunction with glutathione (GSH) to formaldehyde – **Carboxyhemoglobin test may be best measure**		
Styrene	4-vinyl phenol, phenylglycol, mandelic acid, benzoic acid, and hippuric acid **Styrene is thought to accumulate almost exclusively in fat tissue (IARC, 1994)**		0.55 mg/L (blood)
2,5-dimethylfuran	Unknown		

Reference: ACGIH Biological Exposure Indices, 2002 and Lauwerys & Hoet, 1993

Table 2: Cohort Demographic Data

	N	Mean	Median	Range
Age (yrs)	46	31.2	32.5	18-45
Weight (lbs)	46	195.5	195	130-295
Height (inches)	46	70.4	71	61-77

Table 3: Exposure Perception Reported on Questionnaire (N=46)

	Pre- (%)	During (%)	Post- (%)
Depleted Uranium			
None	**98**	57	53
Low	2	**11**	**7**
Medium	0	**7**	0
High	0	0	**2**
Unknown	---	25	38
Chemical Warfare Agents	44	41	35
None	46	32	41
Low	10	7	4
Medium	0	0	0
High	---	**20**	**20**
Unknown			
Passive Smoking			
None	2	7	9
Low	17	24	28
Medium	35	44	50
High	**46**	24	13
Unknown	**----**	0	0
Fuels			
None	15	11	15
Low	39	**63**	**61**
Medium	37	**20**	17
High	9	**6**	2
Unknown	----	**0**	0

Table 4: Ambient Air Sample Averages

Contaminant	Average Concentration (µg/m^3)	Max Concentration (µg/m^3)	StDev Concentraio n (µg/m^3)	n	N
1,2,4-trimethylbenzene	0.35	0.57	0.14	2	16
Benzene	0.57	0.79	0.19	9	16
Carbon Tetrachloride	0.32	0.42	0.08	1	16
Cyclopentane	1.90	8.33	3.59	2	16
Decane	0.55	1.18	0.39	4	16
Ethylbenzene	0.50	1.28	0.45	2	16
Hexane	0.68	2.10	0.80	3	16
m/p-xylene	1.11	3.40	1.29	7	16
Methylcyclopentane	0.38	0.72	0.20	1	16
Methylene chloride	0.84	2.56	0.99	3	16
o-xylene	0.42	0.91	0.28	1	16
Styrene	0.33	0.47	0.11	1	16
Toluene	1.20	1.74	0.49	12	16
Acetone*	7.3	9.5	1.9	5	5

* All samples analyzed by EPA Toxic Organic-17 method except acetone analyzed by EPA Toxic Organic-14 method.
n = number of times detected.
N = number of samples analyzed.

Table 5: OVM Average Air Concentrations

Contaminant	Number of Detections	Average Concentration (ng)*	Range (ng)*	Detection Limit (ng)*
Toluene	**41**	**641.68**	**122 – 3147**	**0.64**
Styrene	**41**	**32.10**	**4 – 157**	**0.019**
m-xylene	**41**	**231.59**	**9 – 6653**	**0.17**
O,p-xylene	**42**	**588.12**	**9 – 6714**	**0.31**
Ethylbenzene	**39**	**39.03**	**10 – 4219**	**0.15**
Benzene	**42**	**22.64**	**4 – 74**	**0.69**

* No average is reported if there are less than five detections of the compound. VOCs present in the Tenax powder were identified and semi-quantitated by using a single point comparison to a known concentration (100 ng) or 21 different VOCs.

Table 6: Blood Benzene Results (n = 46)

	Geometric Mean (ng/mL)	Conf Int (ng/mL)	Range (ng/mL)		p-Value
Pre-	**0.023**	**0.017–0.03**	**0.012-0.20**	**Pre- to During**	**0.07**
During	**0.017**	**0.013–0.023**	**0.012-0.492**	**During to Post**	**0.06**
Post	**0.024**	**0.017–0.033**	**0.012-0.27**	**Pre- to Post**	**0.66**

***Significant at 0.017 (Bonferronni Correction)**
NHANES Reference Range = 0.13 ng/mL

Table 7: Blood Toluene Results (n = 46)

	Geometric Mean (ng/mL)	Conf Int (ng/mL)	Range (ng/mL)		p-Value
Pre-	**0.057**	**0.039–0.084**	**0.013-0.571**	**Pre- to During**	**0.03**
During	**0.089**	**0.061–0.13**	**0.013-0.97**	**During to Post**	**0.01***
Post	**0.049**	**0.031–0.078**	**0.013-0.887**	**Pre- to Post**	**0.55**

***Significant at 0.017 (Bonferronni Correction)**
NHANES Reference Range = 0.52 ng/mL

Table 8: Blood Ethylbenzene Results (n = 46)

	Geometric Mean (ng/mL)	Conf Int (ng/mL)	Range (ng/mL)		p-Value
Pre-	**0.018**	**0.015–0.022**	**0.012-0.082**	**Pre- to During**	**0.01***
During	**0.025**	**0.019–0.032**	**0.012-0.181**	**During to Post**	**0.50**
Post	**0.027**	**0.021–0.035**	**0.012-0.16**	**Pre- to Post**	**<0.001***

***Significant at 0.017 (Bonferronni Correction)**
NHANES Reference Range = 0.11 ng/mL

Table 9: Blood Xylene Results (n = 46)

	Geometric Mean (ng/mL)	Conf Int (ng/mL)	Range (ng/mL)		p-Value
Pre-	**0.081**	**0.07–0.093**	**0.036-0.242**	**Pre- to During**	**0.71**
During	**0.084**	**0.067–0.107**	**0.042-0.502**	**During to Post**	**0.03**
Post	**0.063**	**0.052–0.077**	**0.036-0.468**	**Pre- to Post**	**0.01***

***Significant at 0.017 (Bonferronni Correction)**
NHANES Reference Range m-/p-xylene = 0.37 ng/mL
o-xylene = 0.14 ng/mL

Table 10: Blood Styrene Results (n = 46)

	Geometric Mean (ng/mL)	Conf Int (ng/mL)	Range (ng/mL)		p-Value
Pre-	**0.024**	**0.015–0.11**	**0.036-0.242**	**Pre- to During**	**<0.001***
During	**0.056**	**0.015–0.289**	**0.042-0.502**	**During to Post**	**<0.001***
Post	**0.136**	**0.053–0.432**	**0.036-0.468**	**Pre- to Post**	**<0.001***

***Significant at 0.017 (Bonferronni Correction)**
NHANES Reference Range m-/p-xylene = 0.074 ng/mL

Table 11: 2,5-Dimethylfuran Blood Results (n = 46)

	Geometric Mean (ng/mL)	Conf Int (ng/mL)	Range (ng/mL)		p-Value
Pre-	**0.012**	**0.008–0.016**	**0.006-0.139**	**Pre- to During**	**0.2**
During	**0.014**	**0.009–0.021**	**0.006-0.289**	**During to Post**	**0.3**
Post	**0.012**	**0.008–0.017**	**0.006-0.239**	**Pre- to Post**	**0.9**

***Significant at 0.017 (Bonferronni Correction)**
NHANES Reference Range m-/p-xylene = n/a

Table 12: Non-Parametric Correlations and Linear Regressions between Blood and OVM VOC Sampling Results

Contaminant	**% Detected in Blood**	**% Detected in Air**	**McNemar's p-value***	**Correlation Coefficient**	**p-value***
1,1,1-trichloroethane	2	17	**0.01**		
Benzene	**13**	**91**	**<0.001**		
Carbon Tetrachloride	0	52	**<0.001**		
Chloroform	4	87	**<0.001**		
Ethylbenzene	44	85	**<0.001**	0.2	0.2
Xylene	65	91	**<0.001**	0.35	**0.02**
Styrene	**35**	**89**	**0.01**	0.27	0.1
Tetrachloroethene	**0**	**89**	**<0.001**		
Toluene	85	89	0.2	0.08	0.6

Linear regression values were log transformed to meet more normal distribution.
* McNemar's test with one degree of freedom.
** Significance is based on difference between pre- to post levels.
*** Significance is based on correlation between magnitude of blood and air VOC.

CHAPTER 4: OVERALL DISCUSSION

4.1 Public Health Relevance

As stated previously, analyses of health protection efforts during the Gulf War routinely cite the lack of individual environmental chemical exposure information as a limiting factor in clarifying potential etiologies of illnesses that developed post-deployment (IOM, 1999). Concerns raised by military members and Congress during Operation Enduring Freedom deployments to Afghanistan and neighboring nations have identified the need for accurate monitoring, evaluation and documentation of individual environmental and occupational chemical exposures (FDCH, 2002). Potential exists for similar concerns by Operation Iraqi Freedom veterans. Homeland security threats and protection may also require valid exposure assessment to assess environmental chemical and/or biological agent risk, properly manage these risks, and ultimately prevent illness attributed to such exposures.

Many factors have contributed to the unavailability of deployment individual assessment methods. These are cost, lack of supporting medical infrastructure, lack of medical surveillance systems, and lack of validated medical tests. Therefore, the DoD has not implemented a systematic program to evaluate deployment-related individual exposures even though many independent groups evaluating DoD Force Protection policies and procedures have recommended individual exposure assessment. Current efforts are focused on self-reported exposures captured on questionnaires and intermittent sampling of ambient air, water and soil. Biological testing which confirms or negates exposures to environmental chemicals is a critical element in a comprehensive

environmental and occupational monitoring program. Other essential elements are questionnaire evaluation, environmental area monitoring, and individual environmental monitoring. The MDHEXAS was undertaken on a cohort of Indiana National Guard soldiers deployed to Bosnia Herzegovina from March to August 2002 to determine the need for and to recommend approaches for incorporating biological testing into DoD deployment and force health protection programs.

4.2 General Observations

Prior to the MDHEXAS there had been no statistically valid studies to document during deployment environmental chemical exposures in biological media. It was important to design a valid scientific investigation incorporating all elements of a comprehensive exposure assessment program and meeting all requirements of the USUHS and CDC IRBs. After sampling size was calculated and increased to account for losses to follow up, it became evident that the quantitative evaluation of blood and urine environmental chemicals would only be possible with the collection of greater than 31 pre-, during, and post-deployment blood, urine, and questionnaire samples. This epidemiologic cohort examination was therefore divided into three phases. In the first study phase, soldiers provided informed consent, answered a questionnaire and provided blood and urine samples prior to deployment in March 2002. In the second study phase, soldiers answered a questionnaire, wore an OVM for 24 hours, and provided blood and urine samples during deployment. In the final phase of research, soldiers provided blood and urine samples and answered a final questionnaire post-deployment. The major

objective of all phases of research was to field test the blood and urine environmental chemical exposure sampling techniques.

The results of the MDHEXAS can be generalized to deployed military populations other than the Indiana National Guard unit studied for two reasons:

1. military units are demographically similar to the Indiana National Guard unit studied, and
2. military deployment environments, although varying from the Bosnia Herzegovina environment, do not affect the ability to conduct scientifically and statistically valid biological monitoring pre- and post-deployment.

The MDHEXAS showed that the urine uranium and blood VOC tests were appropriate to document exposures to these environmental exposures during deployment. Although at extremely low-levels, environmental exposures were confirmed for uranium, toluene, ethylbenzene, xylene, and styrene. The results of this study revealed that the chemical agent urine test is not appropriate for deployed environments. Therefore, the MDHEXAS field test and thawing of the chemical agent urine samples led to requesting that the CDC evaluate losses of chemical agent degradation products in urine that has not been frozen.

4.3 Limitations and Uncertainty Analysis

Biological evidence of an increase in environmental chemical exposures leave public health planners with a difficult dilemma. Currently there are no regulatory mechanisms in place to evaluate the increase of biological levels of environmental

chemicals. Additionally, the only mechanisms to evaluate shifts in biological levels of environmental chemicals are:

1. internal comparison of levels pre-, during, and post-exposure,
2. external comparison of levels to the CDC NHANES report of national reference ranges (CDC, 2001 and 2003), and possibly
3. external comparison of levels to available OSHA BEIs.

Truly the science of exposure biomonitoring is in its infancy and requires many more research initiatives for complete understanding and implementation. Because of the newness of the science, this study is limited.

4.3.1 Limitations

Study sample size is always limiting to generalization of research results. A larger cohort would have enabled the investigator to find a more significant difference in the paired samples. A larger cohort would allow for a better explanation of the variability in the data as well. Due to economic constraints, a larger cohort was impossible for this study.

Specific Aim 1 - The field testing of EBs is limited by the available analytical methods and their application. Currently, the blood VOC SPME-MS method has not been full-scale tested at CDC or elsewhere. Although the study attempted to correlate these measures, there is still a need to conduct correlation studies in a more controlled, laboratory setting. Additionally, the need for a military specific external control group is a limitation of the field test portion of this study. This is due to the fact that military populations are generally younger, healthier, and homogeneous in occupational

exposures than the general US population. One result of this research has been that DoD is now aware of the need for a "military NHANES." Finally, this field test is limited by the fact that only one site was sampled. A second sample site would greatly strengthen the results and reliability of the data collected from this research. However, financial and time constraints did not permit a second site.

Specific Aim 2 - The selection of exposure biomarkers is limited by the availability of sensitive and specific biomarkers meeting the criteria established in this study. The criteria used to select exposure biomarkers eligible for field testing were:

(1) sensitive (able to detect chemical when it is present) and specific (able to indicate no chemical when chemical is not present) for common DoD chemicals/exposures,

(2) simple (non-invasive as possible) collection method,

(3) validated analysis method,

(4) availability of environmental and occupational monitoring methods and standards, and

(5) previously used to statistically determine national reference range levels.

Currently, few biomarkers meet these criteria. In the future, research could be expanded to test exposure and affect biomarkers.

Specific Aim 3 - Correlating environmental and occupational samples to exposure biomarkers was limited to those measurements that were taken on the exact same day. Therefore, only VOC individual environmental and biological samples were correlated. This study is limited by the fact that there are not good correlations between environmental and biomarker measurements.

4.3.2 Uncertainty Analysis

As in any applied research effort, there is uncertainty. Uncertainty could stem from inaccurate sampling procedures, improper sample storage and handling, laboratory error, laboratory equipment noise, and data reporting.

As the first full-scale field test prospective cohort using exposure biomarkers to assess individual environmental exposures, it is possible that the method itself is not adequate to measure individual environmental exposures for the following reasons:

1. chemicals being evaluated are metabolized prior to analysis of biological samples, or the chemicals and metabolites cleared from the body prior to sampling,
2. chemical exposures were not significant enough to be measured by this method – sensitivity error, and
3. the chemicals assessed in the laboratory are not the chemicals that individuals were exposed to during deployment – specificity error.

To reduce these three types of uncertainty, three techniques were employed during this research. To address the specificity error, screening measurements were obtained through environmental sampling of the general deployment area along with exposure history, types of operations completed, and prevalence of these compounds in the area. Exposure biomarkers are extremely sensitive and specific measurement techniques, but they are not screening tools. To address the sensitivity error associated with possible metabolic pathways, biological samples were planned to be gathered at the point where exposures are expected (documented through environmental sampling) to be

at the peak. This was impossible to execute effectively during deployment and therefore remains a source of error. VOCs are volatile and non-persistent in the air and the blood. Therefore, blood VOC monitoring has the potential to be impacted by this source of error.

Reliability estimates of reference range concentrations have been published and were considered. However, the NHANES analytical techniques for VOCs, and heavy metals were different than the analytical techniques proposed by this research which could introduce more uncertainty. Additionally, due to the newness of the analytical techniques used for blood and urine analyses, there is a potential for false positive or false negative results. To minimize the false positives, proper control groups (external and possibly internal) and environmental sampling were used.

Correlations of biomarkers to environmental and occupational samples are uncertain because of the lack of an appropriate number of environmental and occupational samples. Had more personal dosimeter measurements been available for the deployment setting or had personal industrial hygiene air sampling been conducted, it may have been possible to conduct further correlations.

4.4 Other Data Collected

As mentioned previously, complete questionnaire data were collected along with biological data for heavy metals and chemical warfare agents. Although not reported in this writing, these results will be analyzed similarly to the urine uranium and blood VOCs are reported in subsequent manuscripts. Finally, the Gulf War Biological Surveillance Initiative sampling data for VOCs will be reported subsequent to these manuscripts.

4.5 Further Research Recommended

This research does not address issues related to military members' attitudes and perceptions toward biological monitoring. Further research generated by this research effort considers gathering information and analyzing for troop reactions and perceptions concerning human blood and urine exposure biomarker methods used to assess toxic chemical exposures during deployments.

Other biomarkers must be tested and validated. Other exposure biomarkers of interest to the DoD are hair and saliva. Hair, which has been used to determine concentrations of heavy metals in an exposed individual, may be an easier method to assess deployment exposures. The field test method proposed by this research should be applied to hair and other biomarkers that are currently in the development stage of research. Finally, development of other health risk assessment methods incorporating biomarkers of effect and using exposure biomarker techniques must be investigated.

Further research is necessary to describe the correlation between EBs and environmental and occupational exposure monitoring. Future research by DoD is needed to describe these relationships for each exposure biomarker which has potential for each exposure biomarker that has the potential for use during military deployments. One potential method to describe these relationships could be animal testing and physiologically based pharmacokinetic modeling. Three programmatic research projects are recommended after completion of this research. They are:

1. development of a military NHANES data base,

2. development of EB standards in humans, and

2. research on the degradation of chemicals in unfrozen urine samples.

Finally, it is necessary to describe the correlation between exposure biomarkers, effect biomarkers, known environmental exposures, and health outcomes. One mechanism to validate the correlation would be to challenge laboratory animals with known concentrations of chemicals and implement exposure and effect biomarker sampling. Effect biomarkers determine the physiologic effect/outcome associated with a known exposure.

4.6 Conclusion

In closing, it is recommended that biomarkers be deployed in conjunction with standard occupational and environmental monitoring methods such as was done in the MDHEXAS. This approach allows more accurate health risk assessment validation. The results of the field test indicate that exposure biomarkers may be a valuable tool to the DoD in exposure and risk assessment from environmental and occupational chemicals. In the MDHEXAS, exposure biomarkers indicate a low-level increased exposure to uranium, styrene, toluene, ethylbenzene and xylene in the deployment environment. The blood styrene levels may indicate that the environment and/or smoking were the cause for increased exposures.

The geometric mean of urine uranium and blood VOC levels were not above U.S. national reference ranges except in the case of styrene which was only slightly above the national reference range. Therefore, adverse health effects greater than seen in the US population are not expected opulation basis due to uranium or VOC exposures to the

Task Force 1-151 Indiana National Guardsman who deployed to Bosnia from March to September 2002 in support of Stabilization Force. Urine uranium and blood VOC analyses appear to provide specific data for individuals that indicate increased exposure to uranium and certain environmental VOCs during deployment. The levels of urine uranium and blood VOCs although different during and post-deployment remained in the "normal" range.

APPENDIX A. Acronym/Symbol Definitions

Acronym/Symbol Definition:

AA -	Atomic Absorption
AFIP -	Armed Forces Institute of Pathology
ATSDR -	Agency for Toxic Substances and Disease Registry
BEI -	Biological Exposure Indices
CBR -	Chemical, Biological, and Radioactive Agents
CDC -	Department of Health and Human Services Centers for Disease Control and Prevention
USACHPPM -	US Army Center for Health Promotion and Preventive Medicine
DoD -	Department of Defense
EPA -	Environmental Protection Agency
FHP -	Force Health Protection
FID -	Flame Ionization Detector
FMP -	Force Medical Protection
FTE -	Full Time Equivalent
FY -	Fiscal Year
GC -	Gas Chromatography
GIS -	Geographical Information System
GPS -	Global Positioning System
GWI -	Gulf War Illness
HPLC -	High Performance Liquid Chromotography
ICAP-AES -	Inductively Coupled Argon Plasma-Atomic Emission Spectroscopy
ICP-MS -	Inductively Coupled Plasma-Mass Spectroscopy
ICRP -	International Commission on Radiological Protection
IRB -	Institutional Review Board
KPA -	Kinetic Phosphoresence Analysis
Maj -	Major
MCEF -	Mixed-Cellulose Ester Membrane Filter in a Styrene Cassette
MDHEXAS -	Military Deployment Human Exposure Assessment Study
mg -	milligram
ml -	milliliter
NBC -	Nuclear, Biological, and Chemical Agents
ng -	nanogram
NHANES -	National Health and Nutrition Examination Survey
OSHA -	Occupational Safety and Health Administration
PRD 5 -	Presidential Review Directive 5, "A National Obligation: Planning for Health Preparedness for and Readjustment of the Military, Veterans, and Their Families after Future Deployments."
ppm -	Parts Per Million
ppt -	Parts Per Trillion
ug -	microgram

USUHS -	Uniformed Services University of the Health Sciences
VOC -	Volatile Organic Compound
XRF -	X-Ray Fluorescence

APPENDIX B. Budget

Detailed Cost Estimate: The total estimated cost of this research is $9,641. Supporting budget worksheets follow ($5,053 will be requested from USUHS, the remaining from USA CHPPM).

Direct Labor Costs --

Maj May will devote 60% effort to the proposed research during PHASE I and 90% effort during PHASE II-III. She will have overall responsibility for supervision of all aspects of the research and of administrative support. In addition, as a formally trained environmental engineer, she will have primary responsibility for designing and overseeing the experimental portions of this research. She has extensive experience in occupational and environmental health risk assessment, program management, and environmental monitoring. Maj May currently receives a stipend from USUHS and will not be seeking compensation for this research.

Additionally, seven government employed Ph.D.s (GS-13 to 15) will dedicate time on this research effort. They are: Dr. Jack Heller (USA CHPPM), Dr. Jim Pirkle (CDC), Dr. David Ashley (CDC), Dr. Vic Kalasinsky (AFIP), Dr. Ben Blount (CDC), Dr. Larry Needham (CDC), and Dr. Gary Gackstetter (USUHS). The Federal Government pays for the time of these individuals. None of their time will be charged in any way to this research contract. It should be noted that these personnel would devote approximately 3 FTEs over the entire 3-year research period. Additional federally funded personnel are a trained phlebotomist and Board Certified Physician. The phlebotomist will draw all blood samples. The physician will serve as the Medical Monitor and will oversee medical care as necessary. Finally, a USUHS Master of Public Health Student will be developing the questionnaire for this research as dictated by the methods section of this protocol. These personnel are not funded by this research effort.

Major Equipment --

All of the major equipment required by this research will be provided by the analytical support facilities at CDC, USA CHPPM, and AFIP. This includes state of the art mass spectrometers and ICP units. A laptop computer was provided by USA CHPPM to support field-deployment data collection activities.

Material, Supplies and Consumables --

PHASE I **– Biomarker Selection Refinement and Field Protocol –** Supplies for this year of research are limited to reproduction ($50). Total Cost = $50. This will be paid for by Maj May’s stipend.

PHASE II **– Data Collection During Deployment** - Reproduction of questionnaires and consent forms will be necessary during this phase of research ($500). Other materials

required by the data collection phase of research are clinical supplies, laboratory fees, and sample shipping costs. The necessary clinical supplies are band aids ($8.66), gauze pads ($6.06), gloves ($33.00), alcohol pads ($19.77), trash bags for infectious waste ($4.60), vacutainer needles ($45.36), vacutainers 10 ml ($210.60), urine specimen cups ($98.75), sharps containers ($121.50), and trash bags ($4.00). Sample shipping is estimated at 500 samples times $2 for a total of $1,000. Laboratory fees will cost $0 for a total of 500 samples. This funding will be waived because of arrangements between the CDC and AFIP with USA CHPPM. Total Cost = $553. These funds will be requested from USUHS/REA.

PHASE III – **Data Analysis and Reporting** – This year costs include reproduction, postage and publication fees. Publications for DoD agency requests will cost $200. Postage will be charged for sending out the fact sheet/data summary to all study participants and pertinent DoD members. Total cost is $0.75 times 100 persons = $75.00. Additionally, the cost of preparing the fact sheet/data summary for each study participant is estimated at approximately $173. Total Cost = $448. These funds will be requested from USUHS/REA.

Analytical Costs --

The CDC has agreed to gratuitously analyze approximated 100 VOC samples and 50 Chemical Weapons samples. Additionally, USA CHPPM has agreed to fund all environmental sampling/analysis. It should be noted that this research will analyze approximately 500 human specimens (300 blood, 50 urine, and an additional 150 urine) for 21 separate chemical analytes using the most advanced analytical methods currently available. $1,500 will be requested from USUHS to pay subjects for the donation of time, blood, and urine.

Travel Costs --

Travel costs for this research were estimated assuming the standard per diem rate of $38.00 per day, car rental $25.00 per day, mileage $30.00 per person, parking $5.00 per day per person, hotel $100.00 per night per person, and airfare $450.00 round trip. **NOTE:** This project is a prospective cohort study of deployed DoD personnel with an approximate 6-month follow-up period. Due to the nature of this study, travel costs are expected to be higher than laboratory-based research study. Additionally, after site selection costs may be adjusted to account for the second year of travel.

PHASE I – **Project Team Meetings** – There is one projected travel during this phase of research. This trip will be to the deploying unit's post prior to deployment to collect the pre-deployment samples. Total cost = $1,320 for one person. These funds will be requested from USA CHPPM.

PHASE II – **Data Collection During Deployment** – One person will need to travel for 15 days to the deployment site to manage pre-deployment data collection by supervising

sampling procedures and preservation protocols are followed ($3,000). Additionally, one person will need to travel to the deployment site two times for 3 days each to oversee during and post-deployment sampling procedures and preservation protocols ($1,320). The deployment site will most likely be Camp McGovern, Bosnia, or South America. Therefore, costs were estimated based upon these locations. Total estimated travel cost = $4,320 for one person. The deployment travel funds will be requested from USUHS ($3,000), and the post-deployment travel funds will be requested from USA CHPPM.

PHASE III – **Data Analysis and Reporting** – There are no projected travel during this phase of research.

Publication and Report Costs --

Publication and report costs will be paid out of Maj May's stipend.

Budget Worksheets:

FY 2001-2003	# of Days	Persons	Airfare	Hotel	Per Diem	Car	Mileage	Parking	Total	Total
Meetings			($450 round)	($100/night)	($38/day/per)	($25/day)	($30/person)	($5/day/per)	Travel	Misc.
Deployment	15	1	$450	$1,500	$570	$375	$30	$75	$3,000	
Site Visit/Sampling	5	1	$450	$500	$190	$125	$30	$25	$1,320	
Site Visit/Sampling	5	1	$450	$500	$190	$125	$30	$25	$1,320	
Reproduction										$500
Laboratory Fees										$0
Clinical Supplies										$553
Sample Shipping										$1000
Subject Payment		50								$1,500
Total										$9,193

FY 2002-2003	Total
	Misc.
Reproduction	$200
Postage, etc.	$248
Total	$448

Clinic Supplies	Supplier	Catalog Number	Quantity Supplied	Number Needed	Number Ordered	Cost Each	Total Cost
Band Aids	Alliance	445	100	200	2	$4.43	$8.86
Gauze Pads	Alliance	3427	100	200	2	$3.03	$6.06
Gloves (non-sterile)	Alliance	6923	50	200	4	$8.25	$33.00
Alcohol Preps	Alliance	3469A	1000	1000	1	$19.77	$19.77
Trash Bag (Infect)	Alliance	6831	100	10	1	$4.60	$4.60
Vacutainer Needle	Alliance	2216	100	200	2	$22.68	$45.36
Vacutainer 10ml	Alliance	0842A	100	1000	10	$21.06	$210.60
Urine Specimen Cup	Alliance		100	500	5	$19.75	$98.75
Sharps Containers	Alliance	5539	20	20	1	$121.50	$121.50
Trash Bags	BX/PX			20	1	$4.00	$4.00
Total							$553

Budget Worksheets (Continued):

Laboratory Fees Analysis	Lab	Samples (quantity)	Cost per sample	Number of Samplings	Total Samples	Total Cost
Blood, VOCs	CDC	50	$0.00	3	150	$0
Urine, Chem Screen	CDC	50	$0.00	1	50	$0
Blood and/or Urine Heavy Metals	USA CHPPM	50	$0.00	3	150	$0
Total					350	$0

Manpower Costs Speciality	# of Speciality	FTE	FTE Pay Rate	Benefits (26% total)	Total
Principal Investigator	1	0.10	$0	$0	$0
Co-Principal Investigator	1	0.60 - 0.90	$0	$0	$0
PhD (GS-14 & 15)	3	0.10	$0	$0	$0
Medical Monitor	1	0.10	$0	$0	$0
PhD Statistician (GS-14)	1	0.10	$0	$0	$0
Laboratory Technicians	3	0.25	$0	$0	$0
Total					$0

Exposure Biomarker Direct-Cost Budget Totals

Fiscal Year	Travel & Misc	Manpower	Total
2000-2001	$0	$0	$0
2001-2002	$9,193	$0	$9,193
2002-2003	$448	$0	$448
Total			$9,641

APPENDIX C. Consultants and Arrangements between Institutions

The US Army CHPPM, CDC, and AFIP letters confirming collaboration with USUHS on this research proposal are attached. USA CHPPM has agreed to work as a liaison between USUHS and the US Army for site selection and logistics of the research. CDC has agreed to perform data analysis for VOCs in blood and a chemical weapons screen in urine. Finally, AFIP and USA CHPPM have agreed to perform data analysis for total and isotopic uranium in urine and heavy metals analysis in blood and/or urine.

APPENDIX D. Cognitive Interview Plan and Explanation

Guidance for the Cognitive Interview was taken from the US Department of Health and Human Services, CDC Working Paper Series, “Cognitive Interviewing and Questionnaire Design: A Training Manual”, Gordon B. Willis Ph.D., March 1994. From the Introduction of this Manual, the purpose of the Cognitive Interview is to reduce error in the questionnaire-based data. The Manual cites, “even if design rules are applied stringently by experts, we still observe significant levels of response error in questionnaire-based data.” Additionally, the Manual cites the traditional field pretest, a small-scale survey conducted before the main survey, as limited because:

1. They tend to focus on the entire survey not each individual question,
2. They tend to call attention to overt, rather than covert issues, and
3. They occur late in the survey development process.

Cognitive Interviewing, as a technique, can help to avoid these issues by:

1. Focusing on the questionnaire instrument,
2. Giving attention to the mental processes that the respondents use to answer survey questions, and
3. Testing survey questions at multiple points in the design process.

The Cognitive Interview took place in October 2001 at an Ordnance Brigade on Aberdeen Proving Ground, MD. The Cognitive Interview required that the volunteer simply answer the italicized questions regarding the survey instrument in a truthful and detailed manner. There were no risks to the volunteers and the only benefit received was food (juice and donuts) provided by the Principal Investigator during the interviews. The following pages provide *(in italics)* the questions that were asked of subjects during the Cognitive Interview. The questions were administered to the subjects verbally using the "probing technique." The "probing technique" is described as follows: after the interviewer asks the survey question, and usually after the subject has answered it, the interviewer asks for other, specific information of the subject (probe further into the basis for the response).

One can see that the Cognitive Interview substantially guided the development of the final questionnaire by observing the differences in the initial questionnaire (Cognitive Interview) and the final questionnaires in Appendix E.

Deployment Biomarkers Questionnaire: Cognitive Interview

This questionnaire is a part of the research study titled, "Exposure Biomarkers in DoD Deployed Personnel and Health Risk Assessment". You should have been fully informed of this research and gave your consent to participate prior to completing this questionnaire. The purpose of this questionnaire is to gather general information on persons providing blood and urine samples for this study. As discussed with you earlier, the blood and urine samples will be analyzed for solvents and chemicals. Blood and urine will **NOT** be analyzed for street drugs. The general information gathered from this questionnaire will be used to determine if any differences exist in chemical levels between different age, gender, smoking, and job categories as well as others as listed below. Please answer these questions to the best of your ability. Thank you for your help.

Do you understand what is meant by the purpose of this questionnaire?

What do you interpret as the purpose of this questionnaire from the above?

What does the term "solvents" mean to you?

What does the term "street drugs" mean to you?

How do you think the information from the study will be used?

1. What is your name? ____
   Last Middle First

2. What is your birthdate? ____
   Month Day Year

3. How much do you weigh? __________ pounds

4. How tall are you? _________________
   feet inches

5. Are you male or female? (Circle One) Male Female

6. Which do you consider your ethnicity? (Circle One Below)

   Caucasian African American Asian

   American Indian Hispanic/Latino Mixed Race

   Other ______________________

What do you think about the categories listed here?

7. Do you use tobacco products? (Circle One) Yes No
If yes, go to question 8. If no, go to question 9.

8. If you use tobacco products, please answer the following:

a. Provide the average number of cigarettes you smoke each day ______________

How do you remember the average number of cigarettes you smoke each day?

b. Have you smoked cigarettes within the past 24 hours? (Circle One)
Yes No

c. Do you smoke cigars? (Circle One) Yes No

What does the term "cigars" mean to you?

d. If yes, estimate how many cigars you smoke each day? ___________________

How do you remember the average number of cigarettes you smoke each day?

e. If yes, have you smoked cigars within the past 24 hours? (Circle One)

Yes No

f. Are you a regular user of chewing tobacco or snuff? (Circle One)
Yes No

What does the term "chewing tobacco or snuff" mean to you?

g. Have you used chewing tobacco or snuff within the past 24 hours?
Yes No

9. Where were you Born? ____
City, State Country

10. What is your home mailing address? ____
Street Number and Name

____
City State Zip

11. When did you come on active duty? ______________________
Month Year

How do you remember when you came on active duty?

12. What was the last occupation you had before coming on active duty? ___________

____

What does the term "last occupation you had before coming on active duty" mean to you?

13. How long did you work at this occupation? __________Years __________Months

How sure are you that this was how long you worked at this occupation?

How do you remember how long you worked at this occupation?

14. What is your current MOS/AOC? ________________________________

15. What is your current Occupation Title? _____________________________________

How did you arrive at this answer?

16. What are your Job Duties (While Not Deployed)? Examples: Tank Driver, Clerk, Aircraft Mechanic, etc.
____

____

Was that easy or hard to answer?

What does the term "job duties" mean to you?

17. Do you have a part-time job outside the military? (Circle One) Yes No

How did you arrive at that answer?

18. If yes, what are your Job Duties in this part-time job? __________________________

____

19. What are your hobbies? ____

What does the term "hobbies" mean to you?

How did you arrive at that answer?

How sure are you that you've listed all your hobbies?

20. Do you work with solvents, paints, or chemicals in your hobbies? (Circle One)
Yes No

What does the term "solvents, paints, or chemicals" mean to you?

21. Have you exercised (run, lift weights, etc.) within the past 4 hours? (Circle One)
Yes No

22. Do you use or are you around Pesticides or Herbicides?(Circle One)
Yes No Not Sure

How sure are you that you use or are around Pesticides or Herbicides?

23. Please circle the level of exposure you think you've had to the following:

Passive Cigarette Smoke	A Lot	Don't Know	A Little
Diesel/Other Fuels	A Lot	Don't Know	A Little
Tent/Heater Fumes	A Lot	Don't Know	A Little
Chemical Weapons Training	A Lot	Don't Know	A Little
Solvents	A Lot	Don't Know	A Little
Paints	A Lot	Don't Know	A Little
Stressful Working Conditions	A Lot	Don't Know	A Little
DEET	A Lot	Don't Know	A Little
Depleted Uranium	A Lot	Don't Know	A Little

Was this hard or easy to answer?

How confident are you that you answered your perceived level of exposure?

24. Did you treat your uniform with Premethrin during this deployment? (Circle One)

Yes No

25. How would you describe your overall general health over the past month? (Circle One)

Excellent Very Good Good Fair Poor

What does the term "general health" mean to you?

Deployment Biomarkers Questionnaire – During Deployment Additional Questions

This is a continuation of the original deployment biomarkers questionnaire that you filled out before leaving on this deployment. The questions in this additional questionnaire are being asked to get a better idea of what you are doing during this deployment.

1. What is your name? ____
Last Middle First

2. What is your birthdate? ____
Month Day Year

3. How much do you weigh? __________ pounds

4. Have your Job Duties during this deployment been different from your non-deployment duties?
(Circle One) Yes No

How sure are you that your duties are or aren't different?

5. If yes, what are your Job Duties during this deployment? Examples: Tank Driver, Clerk, Aircraft Maintenance, etc.
____

____

How do you remember what your job duties are during the deployment?

6. Would you say that your exposures to chemicals have increased during this deployment?
(Circle One) Yes No

What does the term "exposures" mean to you?

7. If yes, which chemicals have you been more exposed to? Please list all that you can.

____

____

How did you determine which chemicals you have been more exposed to?

8. Please circle the level of exposure you think you've had to the following:

Passive Cigarette Smoke	A Lot	Don't Know	A Little

Diesel/Other Fuels	A Lot	Don’t Know	A Little
Tent/Heater Fumes	A Lot	Don’t Know	A Little
Chemical Weapons Training	A Lot	Don’t Know	A Little
Solvents	A Lot	Don’t Know	A Little
Paints	A Lot	Don’t Know	A Little
Stressful Working Conditions	A Lot	Don’t Know	A Little
DEET	A Lot	Don’t Know	A Little
Depleted Uranium	A Lot	Don’t Know	A Little
Drank Non-US, Local Water	A Lot	Don’t Know	A Little
Bathed in Non-US, Local Water	A Lot	Don’t Know	A Little
Chemical Alarm	A Lot	Don’t Know	A Little

9. How do you think your overall general health has been affected by this deployment?
(Circle One) Improved No Change Decreased

How did you arrive at that answer?

10. How do you think your stress level has changed during this deployment?
(Circle One) Increased No Change Decreased

Deployment Biomarkers Questionnaire – After Deployment Additional Questions

1. What is your name? ____
Last Middle First

2. What is your birthdate? ____
Month Day Year

3. How much do you weigh? __________ pounds

4. Have your Job Duties during this deployment been different from your non-deployment duties?
(Circle One) Yes No

5. If yes, what are your Job Duties during this deployment? Examples: Tank Driver, Clerk, Aircraft Maintenance, etc.
____

____

6. Would you say that your exposures to chemicals have increased during this deployment?
(Circle One) Yes No

7. If yes, which chemicals have you been more exposed to? Please list all that you can.

____

____

8. Please circle the level of exposure you think you've had to the following:

Passive Cigarette Smoke	A Lot	Don't Know	A Little
Diesel/Other Fuels	A Lot	Don't Know	A Little
Tent/Heater Fumes	A Lot	Don't Know	A Little
Chemical Weapons Training	A Lot	Don't Know	A Little
Solvents	A Lot	Don't Know	A Little
Paints	A Lot	Don't Know	A Little
Stressful Working Conditions	A Lot	Don't Know	A Little

DEET	A Lot	Don’t Know	A Little
Depleted Uranium	A Lot	Don’t Know	A Little

9. How do you think your overall general health has been affected by this deployment?
(Circle One) Improved No Change Decreased

How did you arrive at that answer?

10. How do you think your stress level has changed during this deployment?
(Circle One) Increased No Change Decreased

How did you arrive at that answer?

APPENDIX E. Deployment Biomarkers Questionnaires

Pre-Deployment Biomarkers Questionnaire

This questionnaire is a part of the research study titled, "EBs in DoD Deployed Personnel and Health Risk Assessment". You should have been fully informed of this research and gave your consent to participate prior to completing this questionnaire. The purpose of this questionnaire is to gather general information on persons providing blood and urine samples for this study. As discussed with you earlier, the blood and urine samples will be analyzed for solvents and chemicals. Blood and urine will **NOT** be analyzed for street drugs. The general information gathered from this questionnaire will be used to determine if any differences exist in chemical levels between different age, gender, smoking, and job categories as well as others as listed below. Please answer these questions to the best of your ability. Thank you for your help.

1. What is your name? ____
   Last First Middle

2. What is your birth date? ____
   Month Day Year

3. How much do you weigh? __________ pounds

4. How tall are you? _________________
   feet inches

5. Are you male or female? (Circle One) Male
Female

6. Which do you consider your ethnicity? (Circle All That Apply)

   Caucasian African American Asian

   Hispanic/Latino American Indian Other____________________

7. Have you ever used tobacco products? (Circle One) Yes No
   If no, please go to question 12.

8. Do you use tobacco products now? (Circle One) Yes No

If yes, go to question 9. If no, go to question 12.

9. Do you smoke cigarettes? (Circle One) Yes No
If no, please go to question 10.

a. How many cigarettes do you smoke each day? _______________

b. Have you smoked cigarettes within the past 24 hours? (Circle One)
Yes No

10. Do you smoke cigars? (Circle One) Yes No
If no, please go to question 11.

a. How many cigars do you smoke each day? ________________

b. Have you smoked cigars within the past 24 hours? (Circle One)
Yes No

11. Do you use chewing tobacco or snuff? (Circle One) Yes No

a. Have you used chewing tobacco or snuff within the past 24 hours?
Yes No

12. Where were you Born? ____
City, State Country

13. When did you begin military service? ______________________
Month Year

14. What was the last occupation you had before coming on active duty? _____________

____

15. How long did you work at this occupation? ___________Years ___________Months

16. What is your current MOS? _________________________________

17. What is the title of your current MOS? ______________________________________

18. What is your secondary MOS? ______________________________

19. What is the title of your secondary MOS?____________________________________

20. What is your present Duty Title? ____

21. If you have been deployed before, please list the deployments in chronological order starting with your most recent deployment (Limit 4):

Place	Dates (From – To)
_____________________________	_________________________
_____________________________	_________________________
_____________________________	_________________________
_____________________________	_________________________

22. Were you deployed to Southwest Asia during Desert Shield/Desert Storm?
(Circle One) Yes No

23. What are your Job Duties (As a Military Member While Not Deployed)? Examples: Tank Driver, Clerk, Infantry Man, Indirect Fire Infantry Man, etc.____________________

____

24. What is your current civilian job? ____

25. What are your Job Duties in your civilian job? _______________________________

____

26. What are your hobbies? ____

27. Do you work with chemicals in your hobbies or other work? (Circle One)

Yes No

28. Have you exercised (run, lift weights, etc.) within the past 4 hours? (Circle One)

Yes No

29. Do you use or are you around Pesticides or Herbicides? (Circle One)

Yes No Not Sure

30. Has your uniform been treated with permethrin (bug repellent)? (Circle One)

Yes No Not Sure

31. Please circle the level of military related exposure you think you've had to the following: (High, Medium, Low, None, and Unknown were the possible responses)

Passive Cigarette Smoke
Fuels/Gasoline
Fumes from Heaters
Chemical Agents
Solvents/Degreasing Chemicals
Paints
Stressful Working Conditions
Bug Spray or Repellent
Depleted Uranium

32. How would you describe your overall general health over the past month? (Circle One)

Excellent Very Good Good Fair Poor

33. Do you want us to mail you the general results of this study? (Circle One)

Yes No

34. If yes, what is the address we could use to mail you this information?

____
Street Number and Name

____
City State Zip

Deployment Biomarkers Questionnaire – During Deployment Additional Questions

This is a continuation of the original deployment biomarkers questionnaire that you filled out before leaving on this deployment. The questions in this additional questionnaire are being asked to get a better idea of what you are doing during this deployment.

1. What is your name? ____
Last First Middle

2. What is your birthdate? ____
Month Day Year

3. How much do you weigh? __________ pounds

4. Have your Job Duties during this deployment been different from your before deployment (civilian) duties?
(Circle One) Yes No

5. If yes, what are your Job Duties during this deployment? Examples: Tank Driver, Clerk, Aircraft Maintenance, etc. ____

____

6. Would you say that your exposures to chemicals have increased during this deployment?
(Circle One) Yes No

7. Please circle the level of exposure you think you've had to the following during this deployment:

Passive Cigarette Smoke	High	Medium	Low	None	Unknown
Fuels/Gasoline	High	Medium	Low	None	Unknown
Tent/Heater Fumes	High	Medium	Low	None	Unknown
Chemical Agents	High	Medium	Low	None	Unknown
Solvents/Degreasing Chemicals	High	Medium	Low	None	Unknown
Paints	High	Medium	Low	None	Unknown
Stressful Working Conditions	High	Medium	Low	None	Unknown
Bug Spray or Repellent	High	Medium	Low	None	Unknown

Depleted Uranium	High	Medium	Low	None	Unknown
Drank Local (off base) Water	High	Medium	Low	None	Unknown
Bathed in Local (off base) Water	High	Medium	Low	None	Unknown
Alerted to Chemical Attack	High	Medium	Low	None	Unknown
Poor Air Quality	High	Medium	Low	None	Unknown
Soil (through digging)	High	Medium	Low	None	Unknown
Dust in the Air	High	Medium	Low	None	Unknown

8. How many times did you put on MOPP gear during this deployment (DO NOT COUNT times that you wore MOPP for training)? ______________________

9. Did you have any problems with the sampler/pouch that you wore on you lapel during operations (twisting, snagging on objects, falling off, discomfort in any way)?
(Circle One) Yes No

10. If you answered YES to question 9, please briefly describe the problem you had:

____

____

11. Did you experience difficulty with the sampler/pouch that you wore on your lapel during the day as you added or removed layers of clothing and protective gear?
(Circle One) Yes No

12. If you answered YES to question 11, please briefly describe the difficulty you experienced:

____

____

13. Do you have any suggestions for improving the Sampler wearability (the one you wore on your lapel) or other comments related to the Individual Passive Chemical Sampler?

____

____

14. Do you think that wearing an Individual Passive Chemical Sampler will improve the military's protection of your health during deployments?

(Circle One) Yes No

15. How do you think your overall general health has been affected by this deployment?

(Circle One) Improved No Change Decreased

16. How do you think your stress level has changed during this deployment?

(Circle One) Increased No Change Decreased

17. What health concerns do you have? ____

____

Post Deployment Biomarkers Questionnaire

This is a continuation of the original deployment biomarkers questionnaire that you filled out before leaving on your deployment. The questions in this additional questionnaire are being asked to get a better idea of what you did during your deployment.

1. What is your name? ____
Last First Middle

2. What is your birthdate? ____
Month Day Year

3. How much do you weigh? __________ pounds

4. Were your Job Duties during this deployment different from your non-deployment duties?
(Circle One) Yes No

5. If yes, what were your Job Duties during this deployment? Examples: Tank Driver, Clerk, Aircraft Maintenance, etc. ____

____

6. Would you say that your exposures to chemicals increased while you were deployed?
(Circle One) Yes No

7. Please circle the level of exposure you think you had to the following while you were deployed:

Passive Cigarette Smoke	High	Medium	Low	None	Unknown
Fuels/Gasoline	High	Medium	Low	None	Unknown
Tent/Heater Fumes	High	Medium	Low	None	Unknown
Chemical Agents	High	Medium	Low	None	Unknown
Solvents/Degreasing Chemicals	High	Medium	Low	None	Unknown
Paints	High	Medium	Low	None	Unknown
Stressful Working Conditions	High	Medium	Low	None	Unknown
Bug Spray or Repellent	High	Medium	Low	None	Unknown

Depleted Uranium	High	Medium	Low	None	Unknown
Drank Local (off base) Water	High	Medium	Low	None	Unknown
Bathed in Local (off base) Water	High	Medium	Low	None	Unknown
Alerted to Chemical Attack	High	Medium	Low	None	Unknown
Poor Air Quality	High	Medium	Low	None	Unknown
Soil (through digging)	High	Medium	Low	None	Unknown
Dust in the Air	High	Medium	Low	None	Unknown

8. How many times did you put on MOPP gear while you were deployed (DO NOT COUNT times that you wore MOPP for training)? ______________________

9. How do you think your overall general health was affected by this deployment?
(Circle One) Improved No Change Decreased

10. How do you think your stress level changed while you were deployed?
(Circle One) Increased No Change Decreased

11. Did you have more colds or illnesses while you were deployed?
(Circle One) Yes No

12. Please list any other concerns you have about chemical exposures from your deployment?

____

____

____

13. While you were deployed, did you begin smoking cigarettes or cigars and/or chewing tobacco?
(Circle One) Yes No

APPENDIX F. Biomarkers During Deployment Data Collection Concept of Operations (CONOPS)

The CONOPS for during deployment data collection is provided as an example of the depth and breath of detail and precision involved in collecting exposure biomarker samples. A CONOPS was written for the pre- and post deployment data collection phases as well. These CONOPS were similar to the Bosnia CONOPS but are not included in this final thesis. The PI maintains all records from all phases and all aspects of study data collection.

BOSNIA BIOMARKERS CONCEPT OF OPERATIONS – SAMPLING STRATEGY (As of 2 Jun 2002)

Table 1. Camp McGovern Ambient Air Chronological Sampling Plan

Camp	McGovern																
Sample Type	PM10			Mod TO1 (TO17)			OVM			TO14	OVM			Blood		Urine	
Duration	24-hour			8-hour						24-hour				VOCs	Metals	Uranium /Metals	Chemical Agents
	S	FB	TB	S	FB	TB	S	FB	TB	S	S	FB	TB	1 Gray Top	2 Purple Top	1 Urine	1 Urine
Day																	
06-Jun (Th)																	
07-Jun (F)	2																
08-Jun (Sa)	2			2	1												
09-Jun (Su)	2																
10-Jun (M)	2													51			51
11-Jun (T)	2			2	1										2 x 51	51	
12-Jun (W)	2																
13-Jun (Th)	2																
14-Jun (F)	2			2	1		3			1	3						
15-Jun (Sa)	2			2	1		3			1	3						
16-Jun (Su)	2			2	1		3			1	3						
17-Jun (M)	2			2	1		3			1	3						
18-Jun (T)	2			2	1		3		1	1	3						
19-Jun (W)	2																
20-Jun (Th)	2																
21-Jun (F)	2			2	1												
22-Jun (Sa)	2																
23-Jun (Su)	2			2	1												
Total	32	0	0	18	9	0	15	0	1	5	15	0	0	51	102	51	51

S- Sample
FB- Field Blank
TB- Trip Blank
OVM – Organic Vapor Monitor

Table 2. Camp Morgan Ambient Air Chronological Sampling Plan

Camp	Morgan																
Sample Type	PM10			Mod TO1 (TO17)			OVM			TO14	OVM			TO14	OVM		
Duration	24-hour						8-hour			24-hour				5-day			
	S	FB	TB	S	FB	TB	S	FB	TB	S	S	FB	TB	S	S	FB	TB
Day																	
06-Jun (Th)																	
07-Jun (F)																	
08-Jun (Sa)	2																
09-Jun (Su)	2			2	1												
10-Jun (M)	2																
11-Jun (T)	2																
12-Jun (W)	2			2	1												
13-Jun (Th)	2																
14-Jun (F)	2			2	1		3			1	3			1	3		
15-Jun (Sa)	2			2	1		3			1	3						
16-Jun (Su)	2			2	1		3			1	3						
17-Jun (M)	2			2	1		3			1	3						
18-Jun (T)	2			2	1		3		1	1	3						
19-Jun (W)	2																
20-Jun (Th)	2																
21-Jun (F)	2			2	1												
22-Jun (Sa)	2																
23-Jun (Su)	2			2	1												
Total	32	0	0	18	9	0	15	1	0	5	15	0	0	1	3	0	0

S- Sample
FB- Field Blank
TB- Trip Blank
OVM – Organic Vapor Monitor

Table 3. Air Sampling Summary

Total Samples	McGovern	Morgan	Total
PM10	32	32	64
TO-17	27	27	54
TO-14	6	6	12
OVM	34	34	68

Table 4. Bosnia Exposure Biomarker Sampling Plan

Camp	McGovern				
Sample Type	OVM	Blood	Blood	Urine	Urine
Contaminant	VOCs	VOCs	Metals	Uranium/Metals	Chemical Agents
Samples	1 OVM	1 Gray Top	2 Purple Top	1 Urine	1 Urine
Day					
1					
2					
3					
4					
5	51				51
6		51	51	51	
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
Ship To					
Technical POC	Batelle	CDC	CHPPM/AFIP	CHPPM/AFIP	CDC

Total	51	51	102	51	51

Table 5. Exposure Biomarker Summary

Total Samples	Total
OVMs	102
Blood (Gray)	51
Blood (Purple)	102
Urine	102

Table 6: **Task Force Eagle Biomarkers Appointments**

Patient ID #	Urine #1 Apt Date	Urine #1 Apt Time	Urine #2 Blood Apt Date	Urine #2 Blood Apt Time
1	10-Jun	0800 hrs	11-Jun	1400 hrs
2	10-Jun	0800 hrs	11-Jun	1400 hrs
3	10-Jun	0800 hrs	11-Jun	1400 hrs
4	10-Jun	0800 hrs	11-Jun	1400 hrs
5	10-Jun	0800 hrs	11-Jun	1400 hrs
6	10-Jun	0800 hrs	11-Jun	1400 hrs
7	10-Jun	0800 hrs	11-Jun	1400 hrs
8	10-Jun	0800 hrs	11-Jun	1400 hrs
9	10-Jun	0800 hrs	11-Jun	1400 hrs
10	10-Jun	0800 hrs	11-Jun	1400 hrs
11	10-Jun	0800 hrs	11-Jun	1400 hrs
12	10-Jun	0800 hrs	11-Jun	1400 hrs
13	10-Jun	0900 hrs	11-Jun	1530 hrs
14	10-Jun	0900 hrs	11-Jun	1530 hrs
15	10-Jun	0900 hrs	11-Jun	1530 hrs
16	10-Jun	0900 hrs	11-Jun	1530 hrs
17	10-Jun	0900 hrs	11-Jun	1530 hrs
18	10-Jun	0900 hrs	11-Jun	1530 hrs
19	10-Jun	0900 hrs	11-Jun	1530 hrs
20	10-Jun	0900 hrs	11-Jun	1530 hrs
21	10-Jun	0900 hrs	11-Jun	1530 hrs
22	10-Jun	0900 hrs	11-Jun	1530 hrs
23	10-Jun	0900 hrs	11-Jun	1530 hrs
24	10-Jun	0900 hrs	11-Jun	1530 hrs
25	10-Jun	0900 hrs	11-Jun	1530 hrs
26	10-Jun	1000 hrs	11-Jun	1700 hrs
27	10-Jun	1000 hrs	11-Jun	1700 hrs
28	10-Jun	1000 hrs	11-Jun	1700 hrs
29	10-Jun	1000 hrs	11-Jun	1700 hrs
30	10-Jun	1000 hrs	11-Jun	1700 hrs
31	10-Jun	1000 hrs	11-Jun	1700 hrs
32	10-Jun	1000 hrs	11-Jun	1700 hrs
33	10-Jun	1000 hrs	11-Jun	1700 hrs
34	10-Jun	1000 hrs	11-Jun	1700 hrs
35	10-Jun	1000 hrs	11-Jun	1700 hrs
36	10-Jun	1000 hrs	11-Jun	1700 hrs
37	10-Jun	1000 hrs	11-Jun	1700 hrs
38	10-Jun	1000 hrs	11-Jun	1700 hrs
39	10-Jun	1000 hrs	11-Jun	1700 hrs
40	10-Jun	1100 hrs	11-Jun	1830 hrs
41	10-Jun	1100 hrs	11-Jun	1830 hrs
42	10-Jun	1100 hrs	11-Jun	1830 hrs
43	10-Jun	1100 hrs	11-Jun	1830 hrs
44	10-Jun	1100 hrs	11-Jun	1830 hrs
45	10-Jun	1100 hrs	11-Jun	1830 hrs
46	10-Jun	1100 hrs	11-Jun	1830 hrs
47	10-Jun	1100 hrs	11-Jun	1830 hrs
48	10-Jun	1100 hrs	11-Jun	1830 hrs
49	10-Jun	1100 hrs	11-Jun	1830 hrs

50	10-Jun	1100 hrs	11-Jun	1830 hrs
51	10-Jun	1100 hrs	11-Jun	1830 hrs

BOSNIA BIOMARKERS DRAFT CONOPS
(As of 2 Jun 2002)

3 Jun: Travel to Frankfurt, Germany
Travel to Ramstein AFB, Germany
Check into Billeting at Vogelweh

4-5 Jun: CHPPM Europe Equipment Check & Shipping Information

6 Jun: Travel to Eagle Base, Tuzla, Bosnia
Meet up with 25th ID
Convoy to Fort McGovern
Check in with Medical, Set up, Team Coordination

7 Jun: Administrative

1. Conduct Site Assessment
    a. Latrine Location
    b. Clinic Location
    c. Request Personnel for Assistance
    d. Environmental Site Assessment (McGovern & Morgan)
2. Conduct Team Meeting to discuss Sampling Strategy
    a. Meet with PA Siade and Phlebotomists
    b. Discuss plan with XO
    c. Dry run Biomonitoring process
3. Set up Clinic Area for Blood & Urine sampling
4. Review all instructions
5. Communicate with Leadership concerning times for sampling
    a. Logistics:
        - Dry Ice
        - Flights
        - Trips to FOB Morgan
    b. Sampling Plan:
    10 Jun 02:
    - 0800 to 1200 hours, Volunteer Urine Sample, OVM (24 hr) Distribution
    - 0600 to 2000 hours, Biomarker Team - Preparation & Aliquoting
    11 Jun 02:
    - 1400 to 2000 hours, Urine Specimen, Blood Draw, Collect 24 hr OVM
    - 0600 to 2200 hours, Biomarker Team - Preparation & Aliquoting

Environmental Sampling Set Up & Site Survey

1. Camp McGovern:
    a. Site Survey and Characterization of Environmental
    b. Contamination

c. GIS coordinates of camp and surrounding area to orient
d. Weather collection
e. Photos
f. Sampling Strategy Finalized

2. FOB Morgan:
   a. Site Survey and Characterization of Environmental
   b. Contamination
   c. GIS coordinates of camp and surrounding area to orient
   d. Weather collection
   e. Photos
   f. Sampling Strategy Finalized
3. Prepare for 24 hour air sampling on Following Day

8 Jun: Biomonitoring

1. Set up Clinic Area for Biomonitoring
2. Finalize Appointments
3. Train Phlebotomists on Procedures
   - Use 91B if Necessary
4. Review OVM instructions
5. Communicate with Clinic Staff concerning times for sampling
   - 0600 to 0800 hours on 10 Jun 02 Biomarker Preparation
   - 0800 to 1200 hours on 10 Jun 02 Urine Draw
   - 1200 to 2000 hours on 10 Jun 02 Aliqoting
   - 0600 to 1200 hours on 11 Jun 02 Biomarker Preparation
   - 1400 to 2000 hours on 11 Jun 02 Phlebotomists, Questionnaire
   - 2000 to 2200 hours on 11 Jun 02 Sample Packaging
   - 0600 to 1200 hours on 12 Jun 02 Shipment Prep and Follow on sampling as necessary

Environmental Sampling

1. Camp McGovern
   a. Set up 24 hour air sampling
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Set up 24 hour air sampling
   b. Air, Water, Soil, Radiation sampling as needed
3. Set up Environmental Sampling for Following Day

9 Jun: Biomonitoring

1. Finalize Clinic Set Up for Biomonitoring
2. Review Appointment List & Phlebotomist Training – Process
3. Finalize Labeling and Urine Aliquoting Techniques

Environmental Sampling

1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
3. Set up Environmental Sampling for Following Day

10 Jun: Biomonitoring

1. Prepare Clinic and Ensure Appointment Times are communicated
2. Collect First Set of Urine Samples
   a. Follow urine collection protocol (attached)
   b. 0800-1200 hours
   c. Chemical Agent Urine Samples
   d. Label Round Urine Cups After Collection
   e. Transport Urine Cup in Biohazard Bag
   f. Aliquot into 2 separate tubes within 6 hours of sample draw and FREEZE (12 ml in each aliquot, maximum, range 10-12 ml)

   NOTE: WEAR POWDER FREE LATEX OR NITRILE GLOVES WHEN ALIQUOTING

   g. Label aliquots with appropriate labels
   h. Record total sample volume, time, and label on Master Log
   i. Prepare for shipment to CDC

2. Pass out VOC Personal Dosimeters (OVMs) with Explanation (see attached protocol)
   a. Record sample time on label and on Master Log
   b. Record Serial Number of OVM on Master Log

3. Set up Urine/Blood/Dosimeter Collection Procedures for Following Day

Environmental Sampling

1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
3. Set up Environmental Sampling for Following Day

11 Jun: Biomonitoring

1. Collect Second Urine Samples
   a. Follow urine collection protocol (attached)

   b. 1400 – 2000 hours

c. Heavy Metals/Uranium Urine Samples
d. Label Square Urine Cup After Collection
e. Transport Urine Cup in Biohazard Bag
f. Record Sample Time on Master Log
g. Keep Samples in Refrigerator (Cool not Frozen)
h. Prepare for Shipment to CHPPM/AFIP

2. Collect Blood Samples
   a. Follow Blood Collection Protocol (attached)
   b. 1400-2000 hours
   c. Use VOC (Gray Top) and 2 Metals (Purple Top) Vacutainers
   d. Label Gray Top and 2 Purple Top Vacutainers before collection
   e. Phlebotomists can use 21, 22 guage, or butterfly needle
   NOTE: AVOID BUTTERFLY NEEDLE UNLESS PERSON WON"T BLEED
   f. Allow skin to dry 15-30 seconds before collecting blood
   g. Fill Gray top tube FIRST with approximately 10 ml of blood (2/3 full)
   h. Fill Purple top tubes as full as possible
   i. Label and record time on Master Log
   j. Mix ALL Vacutainers immediately after collection for:
      - 3 minutes on a rocker, or
      - 30 times by hand
   NOTE: WEAR POWDER FREE LATEX OR NITRILE GLOVES WHEN WORKING WITH BLOOD
   k. Prepare Gray Top (VOCs) blood samples for shipment to CDC
   l. Prepare Purple Top (Metals) blood samples for shipment to CHPPM/AFIP
3. Collect Dosimeters (OVMs)
   a. Double check Serial Number on OVM and Master Log
   b. Record time of collection on Master Log
   c. Place into Refrigerator
   d. Prepare for Shipment to CHPPM Main
4. Complete Questionnaire
   a. Label Questionnaire
   b. Be available to answer questions on the Questionnaire
5. Ship 153 Blood Samples, 102 Urine Aliquots, and 51 Urine Specimens to CHPPM Europe (LT Harrison may Hand Carry)
   a. Ensure all samples are accounted for
   b. Blood should be kept cold (stored in refrigerator until packaged), and packaged with ice packs.
   c. Gray Top (VOCs) blood samples should be re-packaged into original CDC packaging with absorbent material, stabilize in cooler. Place absorbent pad in zip bag with boxed blood samples.
   d. Place blue (absorbent) pads on bottom and around shipper to prevent leakage

f. Urine aliquots must be kept frozen, absorbent pads should be used to prevent leakage

g. Urine Specimens should be packaged with ice packs and absorbent pads to prevent leakage

h. OVMs should be packaged in original packaging and kept cool

i. Ship OVMs to CHPPM main

j. **Place biohazard labels on the outside of all biological shippers, "Noninfectious clinical diagnostic specimens"**

Environmental Sampling

1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
3. Follow Participants to monitor environmental exposures and record job duties
4. Prepare to conduct Environmental Sampling of Participants while working
3. Set up Environmental Sampling for Following Day

12 Jun: Biomonitoring

1. Finalize Blood, Urine, and Dosimeter Packaging and Shipment
2. May need to sample or re-sample if have not obtained all 51 specimens for blood, urine, or OVMs
3. Clinic open from 0600 to 1200 hours
4. LT Harrison travels to Eagle Base & Ramstein AFB, Germany
5. LT Harrison ensures shipment of samples to CDC in Atlanta, CHPPM/AFIP

Environmental Sampling

1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
3. Conduct Environmental Sampling of Participants while working
4. Set up Environmental Sampling for Following Day

13 Jun: Environmental Sampling

1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed

2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
3. Conduct Environmental Sampling of Participants while working
4. Set up Environmental Sampling for Following Day
5. LT Harrison travels to Frankfurt and CHPPM Main/Home

14 Jun: Environmental Sampling

1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
3. Conduct Environmental Sampling of Participants while working
4. Set up Environmental Sampling for Following Day
5. LT Harrison travels to Frankfurt and CHPPM Main/Home

15 Jun: Environmental Sampling

1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
3. Conduct Environmental Sampling of Participants while working
4. Set up Environmental Sampling for Following Day

16 Jun: Environmental Sampling

1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
3. Conduct Environmental Sampling of Participants while working
4. Set up Environmental Sampling for Following Day

17 Jun: Environmental Sampling

1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed

3. Set up Environmental Sampling for Following Day

18 Jun: Environmental Sampling
1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
3. Set up Environmental Sampling for Following Day

19 Jun: Environmental Sampling
1. Camp McGovern
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
2. FOB Morgan
   a. Replace 24 hour air sampling Filters etc…
   b. Air, Water, Soil, Radiation sampling as needed
3. Prepare for shipment of Environmental Samples and Break Down of Equipment

20 Jun: Break down air sampling and other monitoring
Ship Environmental Samples
Pack equipment and prepare for travel

21 Jun: Convoy to Eagle Base, Tuzla, Bosnia
Wait for Transportation to Ramstein AFB, Germany

22 Jun: Travel to Ramstein AFB, Germany

23 Jun: Back Brief CHPPM Europe Commander, Ship Samples

24 Jun: Ship remaining samples

25 Jun: Travel to Frankfurt and Home
Mission Complete ☺

Totals:
14 days of 24 hour Environmental air monitoring
5 days of Occupational monitoring
1 day of personal dosimetry
Suite of Environmental air/water/soil/rad samples
2 urine samples and 1 blood sample

Table 6: Chemical Listing

Toxic Chemical	Environmental Monitoring Method/ Exposure Route	Exposure Biomarker	Exposure Biomarker Analysis Technique	NHANES Reference Range	DoD Use of Chemical
Benzene	Air/Inhalation	Blood	SPME Mass Spec	Yes	Fuels
m-Xylene	Air/Inhalation	Blood	SPME Mass Spec	Yes	Fuels
p-Xylene	Air/Inhalation	Blood	SPME Mass Spec	Yes	Fuels
o-Xylene	Air/Inhalation	Blood	SPME Mass Spec	Yes	Fuels
Ethylbenzene	Air/Inhalation	Blood	SPME Mass Spec	Yes	Fuels
Toluene	Air/Inhalation	Blood	SPME Mass Spec	Yes	Fuels
Methylene Chloride	Air/Inhalation	Blood	SPME Mass Spec	Yes	De-greaser
1,1,1-Trichloroethane	Air/Inhalation	Blood	SPME Mass Spec	Yes	Solvents
1,4-Dichlorobenzene	Air/Inhalation	Blood	SPME Mass Spec	Yes	Solvents
2,5-Dimethylfuran	Air/Inhalation	Blood	SPME Mass Spec	Yes	Combustion
Carbon Tetrachloride	Air/Inhalation	Blood	SPME Mass Spec	Yes	Solvents
Chloroform	Air/Inhalation	Blood	SPME Mass Spec	Yes	Various
Styrene	Air/Inhalation	Blood	SPME Mass Spec	Yes	Various
t-Butyl Methyl Ether	Air/Inhalation	Blood	SPME Mass Spec	Yes	Various
tert-Butyl Alcohol	Air/Inhalation	Blood	SPME Mass Spec	Yes	Solvents
Tetrachloroethene	Air/Inhalation	Blood	SPME Mass Spec	Yes	Solvents
Trichloroethene	Air/Inhalation	Blood	SPME Mass Spec	Yes	Solvents
Nerve agent	Air/Inhalation	Urine	Isotope-Dilution GC-MS-MS	No	Weapon
Sulfur Mustard	Air, Soil, Swipe/ Skin Absorption, Inhalation	Urine	Isotope-Dilution GC-MS-MS	No	Weapon
Total Uranium	Soil/Ingestion & Inhalation	Urine	ICP-Mass Spec	No	Background
Isotopic Uranium	Soil/Ingestion & Inhalation	Urine	ICP-Mass Spec	No	Armor, Penetrators
Cadmium	Air & Soil/Ingestion & Inhalation	Urine/ Blood	ICP-Mass Spec	Yes	Plating, Paints
Chromium	Air & Soil/Ingestion & Inhalation	Urine/ Blood	ICP-Mass Spec	Yes	Plating, Paints
Lead	Air & Soil/Ingestion & Inhalation	Urine/ Blood	ICP-Mass Spec	Yes	Bullets, Paints

Legend: SPME = Solid-Phase Microextraction Technique with Bench top Mass Spectroscopy
Mass Spec = Mass Spectroscopy
ICP-MS = Inductively Coupled Plasma/Mass Spectroscopy
Isotope-Dilution GC-MS-MS = CDC Specific Method using Isotope-Dilution Gas Chromatography-tandem mass spectrometry

BOSNIA BIOMARKERS VOLUNTEER INFORMATION SHEETS

INDIVIDUAL PASSIVE CHEMICAL SAMPLER INSTRUCTIONS

To Use:

1) Remove Sampler from pouch. Save pouch
2) Remove the retaining clip and cover and place them in pouch
3) Reseal the pouch and keep the inside clean
4) Write the sampling start time and date on the reverse label
5) Mount the sampler facing outward on the outside of your uniform
6) Wear sampler for the time period indicated by Sampling Officer

To Return after sampling period is complete:

1) Reseal the sampler with the cover and retaining clip
2) Place resealed sampler in this pouch and reseal the pouch
3) Write the sampling stop time and date on the reverse label
4) Optional: Indicate Unit ID, AO, and observations
5) Return sealed pouch to unit Sampling Officer

Note: Do Not immerse sampler in any liquid

Urine Collection Instructions:

- Remove as much clothing as possible prior to urination and holding the urine collection cup.
- Hands must be washed with soap and water.
- Collection cup should not be opened until just before voiding.
- Person should leave the cap turned up while voiding, then recap the filled container immediately.
- It is important that the inside of the container and the cap not be touched or come into contact with any parts of the body or clothing or external surfaces. Exposure to air should be minimized.
- The participant should deliver the capped specimen immediately to the clinic personnel.

Urine Collection Protocol for Heavy Metals, and Uranium Analysis:

<u>Urine Collection Procedure:</u>

1. Materials Needed per Participant.
   Urine collection cup (120 ml, plastic, sterile)
   Preprinted barcoded label
2. Preparation of Urine Collection Cup for Participant.
   Remove the collection cup with the cap in place from its plastic wrapping being careful not to dislodge the cap or touch the inside of the container or cap.
3. Instructions for Urine Collection.
   The following should be explained to the participant prior to collection:
   - Remove as much clothing as possible prior to urination and holding the urine collection cup.
   - Hands must be washed with soap and water.
   - Collection cup should not be opened until just before voiding.
   - Person should leave the cap turned up while voiding, then recap the filled container immediately.
   - It is important that the inside of the container and the cap not be touched or come into contact with any parts of the body or clothing or external surfaces. Exposure to air should be minimized.
   - The participant should deliver the capped specimen immediately to the clinic personnel.
   - Label urine cup with the correct urine cup barcoded label

<u>Urine Processing Procedure:</u>

1. Materials Needed
   120 ml urine cup with screw cap
   15 ml plastic Falcon tube with screw cap (2 of these tubes)
   Powder-free lab gloves
   Safety glasses
   Boxes with grids to hold 15 ml plastic tubes
   Preprinted barcoded labels (Falcon Tube 1 & 2)
   Freezer (<0° C), refrigerator, or dry ice
2. Special Safety Precautions.
   Universal Precautions
3. Processing (Urine Specimen)
   Wear Powder-free lab gloves, safety glasses, and work under a laboratory hood, if available.
   Using the preprinted labels provided for each participant, affix the labels to the Falcon tubes and add the date collected and the initials of the person preparing the aliquots. Also write the total amount of urine voided into the urine cup onto the special label on the 15 mL tubes.
   Gently swirl the collected specimen in the capped collection container to resuspend any solids.

Aliquot the urine sample into the tubes or vial(s) provided. Fill the tube to the 12ml. DO NOT OVER FILL.
Tightly seal the tube or vial and dispose of the urine container in a biohazard waste container.

4. Urine Storage and Shipping.
   Urine specimens should be refrigerated or frozen as soon as possible after collection and aliquoting. Alternately, samples can be stored in ice chests or on dry ice until delivery at CDC or an alternate storage facility.
   Within 6 hours, the urine samples should be frozen (0° C or less, dry ice is suitable) until shipment.
   If shipment is not immediately possible, please keep samples frozen (0° C or less, dry ice is suitable) until shipping. If samples are shipped to another facility for storage until shipping at a later date, please maintain the chain of custody of the samples and store frozen at 0° C or less.

For shipment instructions, see section below: "Frozen specimen packing and shipping".

<u>Frozen Urine Specimen Packing and Shipping:</u>

1. Materials Needed per Shipper.
   1 styrofoam shipper
   10-12 lbs of dry ice (Frozen only)
   Boxes with grids for 15 ml Falcon tubes
   Safety glasses or eye shield
   Strapping tape
   Gloves for handling dry ice and frozen specimens
   Sheets of bubble-pack packing material
   Federal Express label
   Dry ice label (Frozen only)
   Specimen Shipping List (completed with ID #s and vials or tubes for each number.
   Zip-lock bags
   Urine specimens in 15 ml Falcon Tubes

2. Packing Procedure.
   When packing the shippers, use gloves to handle the dry ice to avoid burns.
   Glasses or an eye shield should also be worn if the dry ice cakes are to be broken into small pieces.
   All boxes with specimens should be taped so the lid does not come off.
   Boxes with samples should be labeled 1 of ___ if more than one box for each type of specimen.
   Whole Blood tubes should be kept cold but **NOT FROZEN** with cold packs.
   Urine samples should remain **frozen with dry ice**.
   Place each box of specimens in a zip lock bag with an absorbent pad.

Place the specimens in the cooler.
Label shippers appropriately with properly labels to indicate dry ice, etc.
The amount of dry ice should be approximately 1 lb. or more for every 2 hours of transit.
Samples are called “Non Infectious DIAGNOSTIC SPECIMENS”

SHIPPING LIST

A collection log is provided to record samples that are collected. Please mark the appropriate spaces indicating which aliquots were collected, date collected and any problems that were encountered in collection, storage, or shipping. Include a copy of this log when shipping specimens to CDC.

SHIPPING PROCEDURE

1a. **Blood Tubes**: There are boxes with grids that are provided for storage and shipping of the individual tubes. Place each box of specimens in a zip lock bag along with some white absorbent pads. Include a copy of the collection log. Fill the shipper (cardboard box with inner styrofoam box) with ice packs, cover with the styrofoam lid and tape down the cardboard outer flaps.

1a. **Urine Tubes**: There are boxes with grids that are provided for storage and shipping of the individual tubes. Place each box of specimens in a zip lock bag along with some white absorbent pads. Include a copy of the collection log. Fill the shipper (cardboard box with inner styrofoam box) with dry ice (at least 1 lb for every 2 hours of transit), cover with the styrofoam lid and tape down the cardboard outer flaps. Place a dry ice label on the outside of the container and write in the amount of dry ice in the shipper.

2. Ship to the following address:

   CHPPM MCHB-TS-HER
   5158 Blackhawk Rd, Bldg E-1675
   Aberdeen Proving Ground, MD 21010-5403

3. Please email jack.heller@apg.amedd.army.mil or call (410) 436-5243 on the day the shipment is made. Also, if any questions arise, please call the above number.

Collection of Blood Samples for Measurement of Volatile Organic Compounds at the Centers for Disease Control

Previous studies of VOCs indicate that their half-life in human blood is extremely short. In many cases, values between 10 and 30 minutes are considered to be the best estimates for these half-lives in cases of acute exposure. Because VOCs do not reside long in the body, special sample collection considerations are necessary. Except in cases of extremely high exposure, sampling of blood after as much as 2 days after removal from exposure may not indicate abnormal levels in the blood. Of course the length of time after exposure for which useful samples can still be obtained will vary with the level of exposure. It is therefore suggested that samples be obtained either before removal from exposure or as quickly after this time as possible.

Vacutainer tubes obtained from commercial sources contain VOC contamination which can greatly interfere with the ability to obtain analytical results which are indicative of the degree of exposure. Tubes which were obtained commercially have been specially modified at CDC so that they no longer contain measurable levels of most VOCs. It is absolutely imperative that these tubes be used for all sample collections to insure a viable sample. These tubes will be supplied for all VOC studies.

The anticoagulant used in the CDC prepared tubes is a mixture of sodium oxalate and sodium fluoride. This anticoagulant is chiefly intended to stop metabolism so that VOC levels do not change appreciably during storage. This mixture's ability to prevent clotting of blood is not as great as many other anticoagulants. Thus, once samples have been collected, they must be mixed thoroughly to allow the complete distribution of the anticoagulant. If a blood mixer is available, samples should be placed on this mixer for at least 3 minutes. If a mixer is not available, the blood can be mixed by hand approximately 30 times to completely mix the anticoagulant into the blood sample.

Isopropanol used to disinfect the venipuncture site has resulted in interferences in the analytical measurement by introduction of this compound into samples. This can be easily prevented by swabbing the site with a dry gauze bandage and allowing the site to dry for 5 - 10 seconds after wiping with isopropanol.

Since VOCs are highly volatile, care must be taken to insure that samples are kept at refrigerator temperatures during storage and shipment. All samples should be placed on wet ice or into a refrigerator within 30 minutes of sample collection. In addition, samples should be shipped with enough wet ice or equivalent cooling material to insure that the samples will remain cool throughout the shipment process. Samples should be shipped to insure that they will arrive at CDC on normal business days to insure their proper processing upon arrival. Samples should not be frozen or stored at freezer temperatures at any time during sample collection and shipment. Preliminary experiments have indicated that the concentration of some volatile analytes changes over sample storage time. Therefore, the samples should be shipped within 1 - 2 days of collection so that they can be analyzed within 8 weeks of collection.

Once the samples are injected into the sampling device it is not possible to recover these samples and reanalyze them. On occasion, problems do arise during the measurement phase and it is not possible to make these determinations. Thus, the samples which have been committed to analysis on this day cannot be analyzed. For this reason it is important that backup samples be provided for each subject. Whenever possible 10 mL of blood should be collected on each individual to provide 2-5 mL samples.

Freeze packs must be included in specimen packages. Please freeze these and include in the shipment to keep the samples cold during transport. All samples should be sent by overnight carrier so that they will arrive at CDC still cold. Extra vacutainers are in case of breakage or loss of vacuum.

Do not include any personal identifying information (name) on the vacutainer tube, but label each tube so that they can be identified later. Be certain to carefully record a description of the sample collected on the sample transmittal sheet next to the identifying number. Include a copy of the transmittal sheet in the sample shipment and retain a copy for your own records. The sample transmittal sheet will be retained by the supervisor and not be available to the analyst. It is extremely important that this transmittal sheet be accurate since it will be the only link between sample numbers and the sample description.

Important points to remember:

1) Allow isopropanol to evaporate from the arm

2) Mix sample well after collected

3) Keep sample at refrigerator temperatures, but do <u>not</u> freeze

4) Do <u>not</u> open sample. Do <u>not</u> remove whole cells. Ship as collected.

5) Ship samples to arrive at CDC on normal business day

6) Whenever possible 10-mL samples should be collected

7) Any questions about this procedure or the shipment of samples can be addressed to Dr. Ben Blount (770) 488-7894.

Procedure

1. Have the following items on hand and available for use.
   - Tourniquet
   - Alcohol disinfectant swabs
   - Gauze bandages
   - 22g vacutainer needle
   - Vacutainer needle holder
   - CDC-supplied grey-top vacutainer tube
   - Bandaid
   - Blood mixer (if available)
   - Sharps disposal container for used needles
   - CDC-supplied sample labels
   - Ice or Refrigerator for storage
2. Tie the tourniquet onto the upper arm so that it can be quickly released with one hand.
3. Swab the venipuncture area with alcohol swabs.
4. Wipe off excess alcohol with the gauze bandages.
5. Allow to air dry for 5 - 10 seconds.
6. Puncture the vein.
7. Attach the vacutainer.
8. After blood flow is established, loosen the tourniquet.
9. Allow the vacutainer to fill to within 1 to 2 cm of the top of the tube.
10. Remove the vacutainer and place on the blood mixer if available.
11. Withdraw the needle and dispose of in the sharps disposal container.
12. Place pressure on the venipuncture site.
13. If mixing by hand rotate the vacutainer at least 30 times to insure good distribution of anticoagulant throughout the blood.
14. Attach the sample label to the side of the vacutainer
15. Record the sample number (from the label) and sample description on the transmittal sheet.
16. Within 30 min place the samples either on wet ice or within a refrigerator.
17. Include the transmittal sheet in the sample shipping container.
18. Ship samples by overnight carrier in insulated containers along with enough ice or ice packs so that the temperature can be maintained during the shipping process. Samples should be shipped to the following address so that they will arrive on a normal working day (Monday - Friday, non-Federal Holidays). If there is any question about their arrival on a normal working day, hold the samples until this question is answered.

FedEx:
Dr. Ben Blount
Centers for Disease Control and Prevention
Bldg 17 Loading Dock
4770 Buford Highway, NE
Atlanta, GA 30341-3724

Express Mail:
Dr. Ben Blount
Mailstop F-19
CDC
4770 Buford Highway, NE
Atlanta, GA 30341-3724

APPENDIX G. Biomarkers Informed Consent Document

INFORMED CONSENT DOCUMENT

STUDY TITLE: EXPOSURE BIOMARKERS AS ENVIRONMENTAL SURVEILLANCE TOOLS

PRINCIPAL INVESTIGATOR: LISA M. MAY, USAFR, BSC, EIT

INTRODUCTION:

You are being asked to take part in a research study. Before you decide to be a part of this research study, you need to understand the risks and benefits so that you can make an informed decision. This is known as informed consent.

This consent form provides information about the research study, which has been explained to you. Once you understand the study and the tests it requires, you will be asked to sign this form if you want to take part in the study. Your decision to take part in the study is voluntary. This means that you are free to choose if you will take part in the study.

PURPOSE AND PROCEDURES:

The Department of Preventive Medicine and Biometrics of the Uniformed Services University of the Health Sciences (USUHS) and the US Army Center for Health Promotion and Preventive Medicine (CHPPM) are carrying out a research study to find out if chemicals in the environment at a deployment site can be measured in the blood and urine of deployed persons. Harmful levels of chemicals are not expected during your deployment. The blood and urine tests selected by USUHS and CHPPM are able to detect chemicals in the blood and urine at extremely low levels. To complete this research, USUHS will be gathering blood and urine samples from 50 persons preparing to deploy overseas. These samples will be collected at three different times: before, during, and after deployment. There are a number of steps to this research:

1. Before you decided to participate in this study, you should have been briefed on the study and received a fact sheet detailing the study and the points of contact for the study. About one week before deployment, we would like to get a sample of your blood (one needle stick and two to three tubes each containing about three tablespoons) and urine for laboratory tests. The blood samples will be taken exactly as it is done in a medical treatment facility and will be completed by a military medical technician. The urine samples will also be taken in a medical manner - this is not a drug-screening test and you will not be observed while giving your urine sample - it is private. When you give the first set of blood and urine samples, you will be paid $10.00.

2. On that same day we would like to ask you to complete a questionnaire (about 3 pages) aimed at getting information on occupation and hobbies.

3. During your deployment, we would like to get another sample of your blood (one needle stick and two to three tubes each containing about three tablespoons) and urine for the same laboratory tests performed on your first samples.

4. During your deployment on the same day that you give blood and urine samples, we would like you to fill out a one-page questionnaire. This questionnaire will ask you specifically what you've been doing during your deployment. When you give the set of blood and urine samples during the deployment, you will not be given any money.

5. After your deployment, we would like to get the final blood sample (one needle stick and two to three tubes each containing about three tablespoons) and urine sample. After deployment, when you give your final set of blood and urine samples, we will give you $20.00 – total amount for giving all three sets of samples is $30.00.

6. Finally, we would like you to fill out a one-page questionnaire after your deployment that will ask you about any potential exposures during your deployment.

All samples and questionnaires will be kept confidential. After the study, all blood and urine samples and any excess blood or urine will be destroyed. General results from this study will be sent to you if you ask for them on the informed consent form. Specific results cannot be reported to you because this type of information is new and there is no current way to interpret the data's meaning.

BENEFITS:

There are no direct benefits to your participating in this study. However, by participating in this study, you are helping the military medical community to learn the best way to measure whether deployed troops have been exposed to toxic chemicals above what would normally be expected in the U.S. national population.

RISKS:

There are two identified, potential risks to you from enrolling in this study. The first risk is that of a swelling, redness, bruising, and slight pain at the site where the needle is inserted at the time of a blood draw. These effects may last up to a day or two after the procedure. The blood sample that we will take will be only a small tube of blood, similar to that given for your annual physical exam. If you know that you have had problems in the past, please let someone in the study know.

The second risk is that you may worry about the results of these tests since we have no current way to interpret their meaning. This is a research study and, therefore, we are attempting to better understand these samples. If you request general information, please read the study information provided to you and contact your physician with any specific questions you may have. It is possible that this knowledge could cause you stress, in which case please contact Ms. May (301)-295-9768, Dr. Heller (410)-436-5243, or your physician immediately with your concerns.

RIGHT TO WITHDRAW FROM THE STUDY:

You may decide to stop taking part in this study at any time. Your health care and relations with the faculty, staff and administrators at USUHS or with your commanding officer will not change in any way if you decide to end your participation in the study. To terminate participation in this study, please contact Dr. Jack Heller at (410) 436-5243 or Ms. Lisa May at (301) 295-9768, e-mail: jack.heller@apg.amedd.army.mil or lmay@usuhs.mil and you will be withdrawn immediately. Terminating your participation in this study will not impact your military standing concerning promotions or other job related activities.

RECOURSE IN THE EVENT OF INJURY:

This study should not entail any physical or mental risk beyond those described above. We do not expect complications to occur, but if, for any reason you feel that continuing this study would constitute a hardship for you, we will end your participation in the study.

DoD will provide medical care at government facilities for any DoD eligible (active duty, dependents, and retired military) for injury or illness resulting from participation in this research. Such care may be available through judicial avenues to non-active duty research participants if they are injured through the negligence (fault) of the Government. Such care may not be available if you become no longer eligible for military health care.

If at any time you believe you have suffered an injury or illness as a result of participating in this research project, you should contact Dr. Jack Heller at (410) 436-5243, or the Office of Research at the Uniformed Services University of the Health Sciences, Bethesda, MD 20814 at (301) 295-3303. This office can review the matter with you, provide information about your rights as a subject, and may be able to identify resources available to you. Information about judicial avenues of compensation is available from the University's General Counsel at (301) 295-3028.

PRIVACY AND CONFIDENTIALITY:

The results of this research study will be given to the U.S. Army Center for Health Promotion and Preventive Medicine and may be asked for by the US Department of Health and Human Services. None of the information given to these people will

contain names or other information linking any results to you specifically. Records from this study will not use your name or identify you personally.

All information that you provide as a part of this study will be confidential and will be protected to the fullest extent of the law. Information that you provide and other records related to this study will be kept private, accessible only to those persons directly involved in conducting this study and members of the USUHS Institutional Review Board and other Federal agencies who provide oversight for human use protection. All questionnaires and forms will be kept in a restricted access, locked cabinet while not in use. The questionnaires will be numbered to maintain anonymity and will not contain any identifying information. Only the project officer in charge will have access to the code. However, please be advised that under Federal Law, a military member's confidentiality cannot be strictly guaranteed. To enhance your privacy of the answers that you provide, data from questionnaires will be entered into a database in which individual responses are not identified. After verification of the database information, the hard copy of the questionnaires containing identifiers will be shredded.

All biological samples, blood and urine, will be destroyed after the analysis is completed. The biological samples you give will not be used for anything other than the determination of volatile organic compounds in your blood, metals in your blood and urine, uranium in your urine, and chemical warfare agents in your urine.

QUESTIONS:

If you have any questions about this research study, you should contact Dr. Jack Heller at (410) 436-5243 during a workday or Lisa May (301) 295-9768 at night and on weekends. If Dr. Heller or Ms. May are not responsive, please call Dr. Coleen Weese at (410) 436-2714. If you have any questions about your rights as a research subject, you should call the Director of Research Programs in the Office of Research at the Uniformed Services University of the Health Sciences at (301) 295-3303. This person is your representative and has no connection to the individuals conducting this study.

☐ Please check this box if you want a copy of the General Interpretation of Results from this research study sent to your address.

To obtain specific interpretation of these results, you must contact your physician.

SIGNATURES:

By signing this consent form you are agreeing that the study has been explained to you and that you understand the study. You are signing that you agree to take part in the study. You will be given a copy of this consent form.

I give my permission for representatives of the USUHS Exposure Biomarker Research Program to take three samples of my blood and three samples of my urine for the purposes of medical research. This program was explained to me and I understand that I agree to take part in the study with the ability to withdraw at any time.

NAME: (please print) ____

ADDRESS: (if results are requested) __________________________________

____

SIGNATURE: ________________________________ DATE: ___________

WITNESS: __________________________________ DATE: ___________

INVESTIGATORS STATEMENT:

I certify that the research study has been explained to the above individual by me or my research staff and that the individual understands the nature and purpose, the possible risks and benefits associated with taking part in the research study. Any questions that have been raised have been answered.

INVESTIGATOR: ________________________________

DATE: _______________

CO-INVESTIGATOR: ______________________________

DATE: _______________

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